

ATG GTA CGT AGC TCC TCT CGC ACT CCG TCC GAT AAG CCG GTT GCT  
M V R S S S R T P S D K P V A

CAT GTA GTT GCT AAC CCT CAG GCA GAA GGT CAG CTG CAG TGG CTG  
H V V A N P Q A E G Q L Q W L

AAC CGT CGC GCT AAC GCC CTG CTG GCA AAC GGC GTT GAG CTC CGT  
N R R A N A L L A N G V E L R

---

GAT AAG CAG CTC GTG GTA CCT TCT GAA GGT CTG TAC CTG ATC TAT  
D N Q L V V P S E G L Y L I Y

TCT CAA GTA CTG TTC AAG GGT CAG GGC TGC CCG TCG ACT CAT GTT  
S Q V L F K G Q G C P S T H V

CTG CTG ACT CAC ACC ATC AGC CGT ATT GCT GTA TCT TAC CAG ACC  
L L T H T I S R I A V S Y Q T

AAA GTT AAC CTG CTG AGC GCT ATC AAG TCT CCG TGC CAG CGT GAA  
K V N L L S A I K S P C Q R E

ACT CCC GAG GGT GCA GAA GCG AAA CCA TGG TAT GAA CCG ATC TAC  
T P E G A E A K P W Y E P I Y

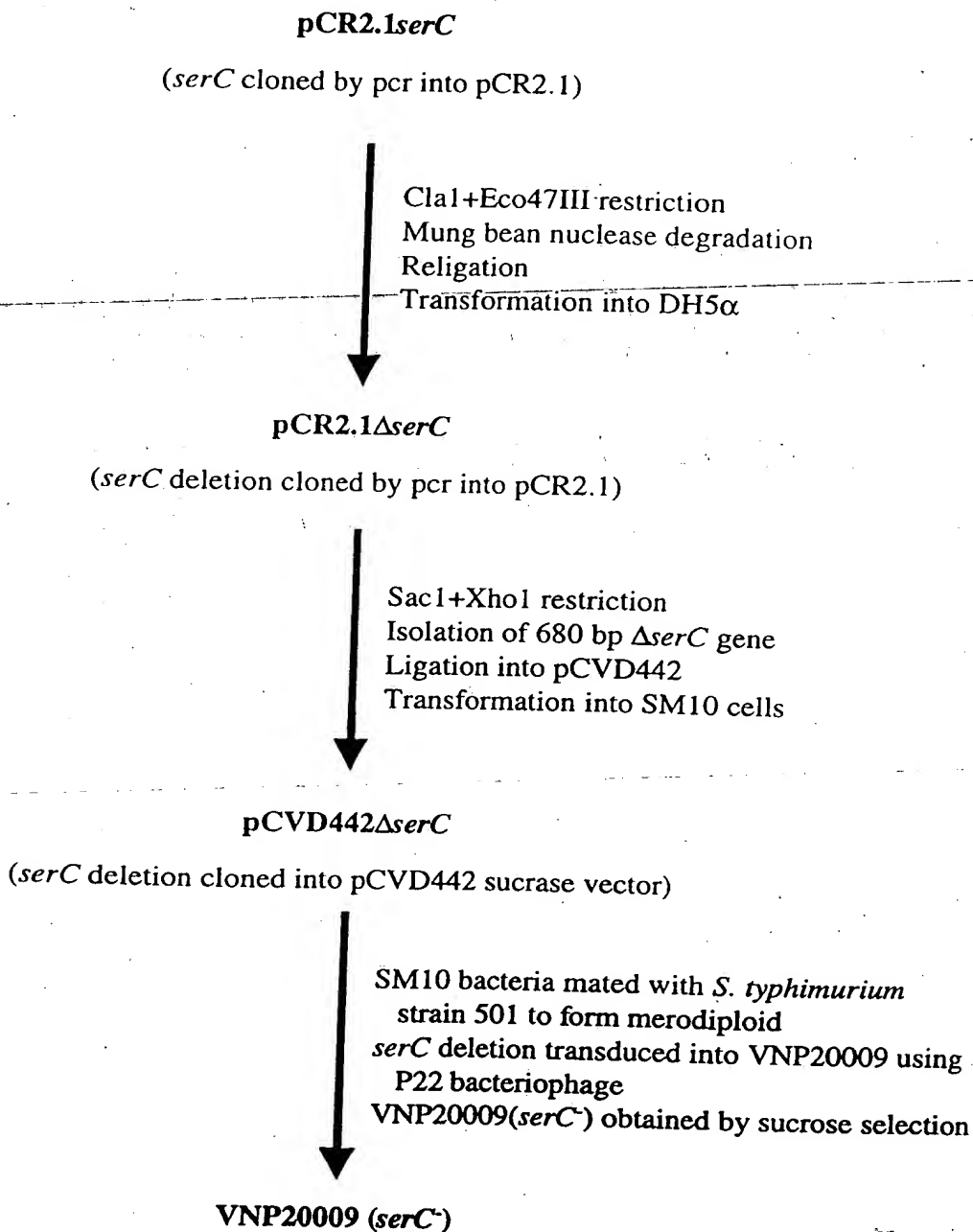
CTG GGT GGC GTA TTT CAA CTG GAG AAA GGT GAC CGT CTG TCC GCA  
L G G V F Q L E K G D R L S A

GAA ATC AAC CGT CCT GAC TAT CTA GAT TTC GCT GAA TCT GGC CAG  
E I N R P D Y L D F A E S G Q

GTG TAC TTC GGT ATT ATC GCA CTG TAA  
V Y F G I I A L \*

FIG. 1

## Derivation of the VNP20009(*serC*<sup>-</sup>) strain.

**FIG. 2**

# Quantitation of TNF $\alpha$ expression by pTS-BrpTNF $\alpha$ Clone 2.

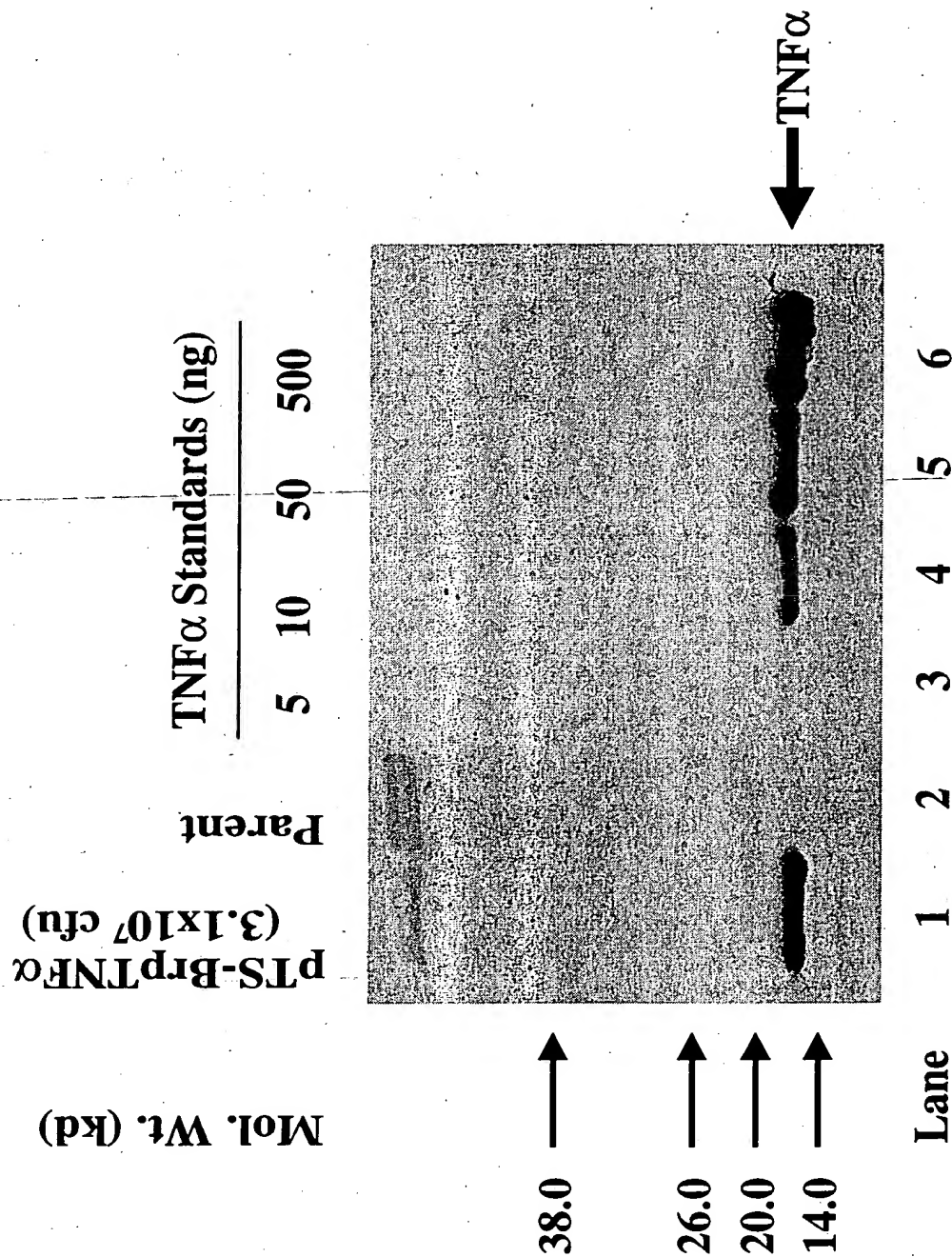


FIG. 3

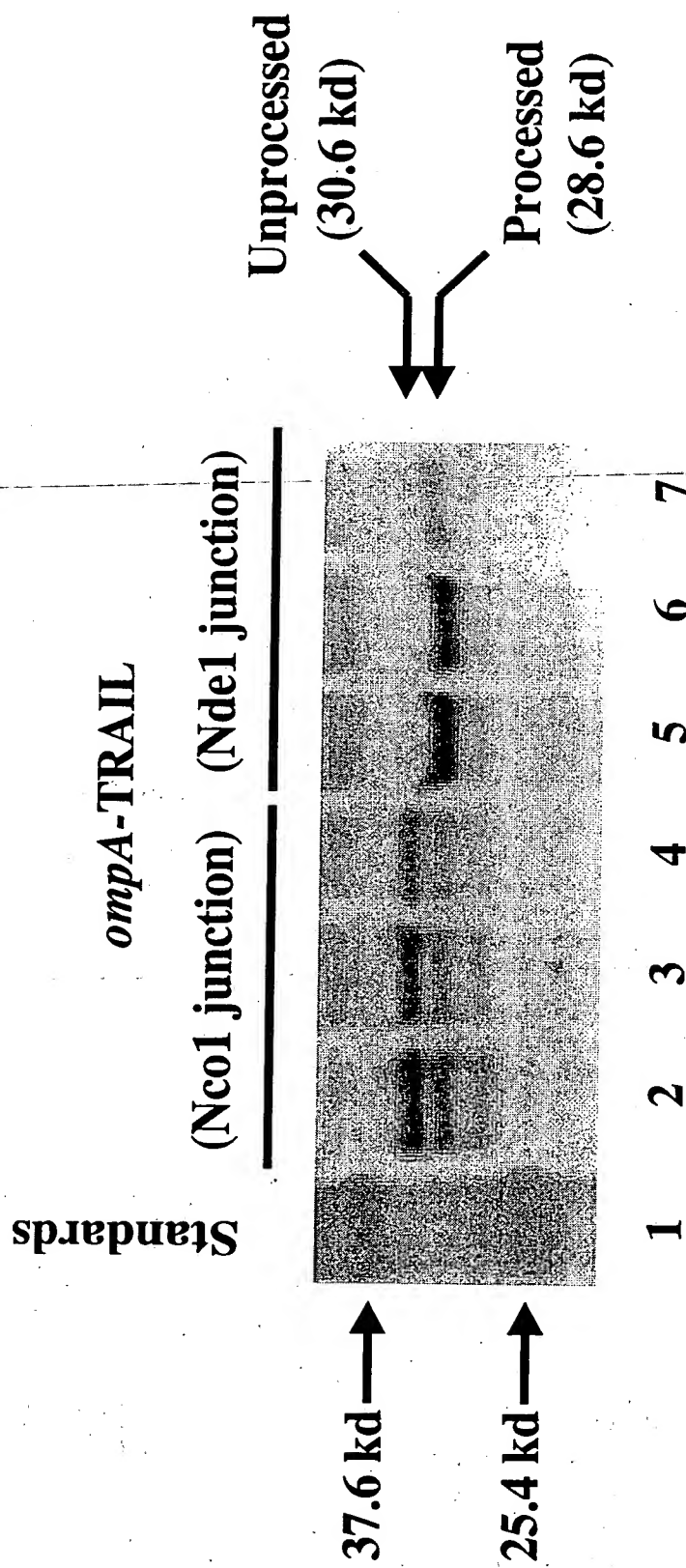
ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC  
 M K K T A I A I A V A L A G F  
 GCT ACC GTA GCG CAG GCC CAT ATG GTA CGT AGC TCC TCT CGC ACT  
 A T V A Q A H M V R S S S R T  
 CCG TCC GAT AAG CCG GTT GCT CAT GTA GTT GCT AAC CCT CAG GCA  
 P S D K P V A H V V A N P Q A  
 GAA GGT CAG CTG GAG TGG CTG AAC CGT CGC GCT AAC GCC CTG CTG  
 E G Q L Q W L N R R A N A L L  
 GCA AAC GGC GTT GAG CTC CGT GAT AAC CAG CTC GTG GTA CCT TCT  
 A N G V E L R D N Q L V V P S  
 GAA GGT CTG TAC CTG ATC TAT TCT CAA GTA CTG TTC AAG GGT CAG  
 E G L Y L I Y S Q V L F K G Q  
 GGC TGC CCG TCG ACT CAT GTT CTG CTG ACT CAC ACC ATC AGC CGT  
 G C P S T H V L L T H T I S R  
 ATT GCT GTA TCT TAC CAG ACC AAA GTT AAC CTG CTG AGC GCT ATC  
 I A V S Y Q T K V N L L S A I  
 AAG TCT CCG TGC CAG CGT GAA ACT CCC GAG GGT GCA GAA GCG AAA  
 K S P C Q R E T P E G A E A K  
 CCA TGG TAT GAA CCG ATC TAC CTG GGT GGC GTA TTT CAA CTG GAG  
 P W Y E P I Y L G G V F Q L E  
 AAA GGT GAC CGT CTG TCC GCA GAA ATC AAC CGT CCT GAC TAT CTA  
 K G D R L S A E I N R P D Y L  
 GAT TTC GCT GAA TCT GGC CAG GTG TAC TTC GGT ATT ATC GCA CTG  
 D F A E S G Q V Y F G I I A L

TAA

\*

FIG. 4

**Expression and processing of a *trc* promoter-driven *ompA*-TRAIL fusion gene product in JM109 bacteria.**



**FIG. 5**

ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC  
 M K K T A I A I A V A L A G F  
  
 GCT ACC GTA GCG CAG GCC CAT ATG GCT AAC GAG CTG AAG CAG ATG  
 A T V A Q A H M A N E L K Q M  
  
 CAG GAC AAG TAC TCC AAA AGT GGC ATT GCT TGT TTC TTA AAA GAA  
 Q D K Y S K S G I A C F L K E  
  
 GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA GAG AGT ATG AAC AGC  
 D D S Y W D P N D E E S M N S  
  
 CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG CTC GTT AGA AAG  
 P C W Q V K W Q L R Q L V R K  
  
 ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA  
 M I L R T S E E T I S T V Q E  
  
 AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG  
 K Q Q N I S P L V R E R G P Q  
  
 AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA  
 R V A A H I T G T R G R S N T  
  
 TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA  
 L S S P N S K N E K A L G R K  
  
 ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC  
 I N S W E S S R S G H S F L S  
  
 AAC TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG  
 N L H L R N G E L V I H E K G  
  
 TTT TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA  
 F Y Y I Y S Q T Y F R F Q E E  
  
 ATA AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT  
 I K E N T K N D K Q M V Q Y I  
  
 TAC AAA TAC ACA AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT  
 Y K Y T S Y P D P I L L M K S  
  
 GCT AGA AAT AGT TGT TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT  
 A R N S C W S K D A E Y G L Y  
  
 TCC ATC TAT CAA GGG GGA ATA TTT GAG CTT AAG GAA AAT GAC AGA  
 S I Y Q G G I F E L K E N D R  
  
 ATT TTT GTT TCT GTA ACA AAT GAG CAC TTG ATA GAC ATG GAC CAT  
 I F V S V T N E H L I D M D H  
  
 GAA GCC AGT TTT TTC GGG GCC TTT TTA GTT GGC TAA  
 E A S F F G A F L V G \*

FIG. 6

# Expression and processing of a *trc* promoter-driven *ompA*-TNF $\alpha$ fusion gene product in JM109 bacteria.

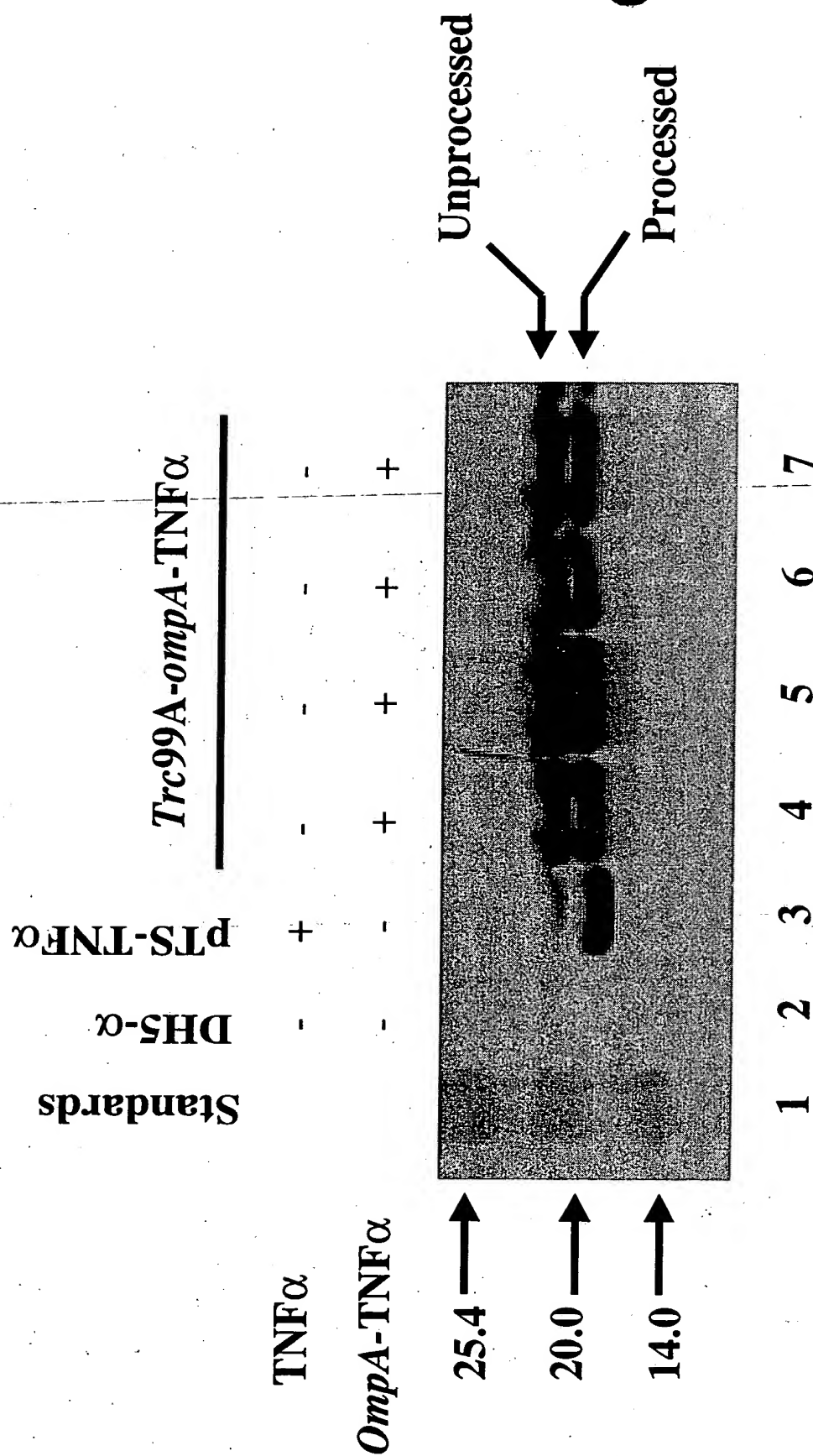


FIG. 7

ATG AAA AAG ACG GCT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG  
 M K K T A L A L L L L L L A L  
 ACT AGT GTA GCG CAG GCC GCT CCT ACT AGC TCG AGC ACT AAG AAA  
 T S V A Q A A P T S S S T K K  
 ACT CAA CTG CAA TTG GAG CAT CTG CTG CTG GAT CTG CAG ATG ATT  
 T Q L Q L E H L L L D L Q M I  
 CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG  
 L N G I N N Y K N P K L T R M  
 CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA  
 L T F K F Y M P K K A T E L K  
 CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA  
 H L Q C L E E E L K P L E E V  
 CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC  
 L N L A Q S K N F H L R P R D  
 CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC  
 L I S N I N V I V L E L K G S  
 GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT  
 E T T F M C E Y A D E T A T I  
 GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT  
 V E F L N R W I T F A Q S I I  
 AGC ACG TTA ACT TAA  
 S T L T \*

FIG. 8



# Periplasmic localization and processing of *ompA*-IL2 fusion proteins.

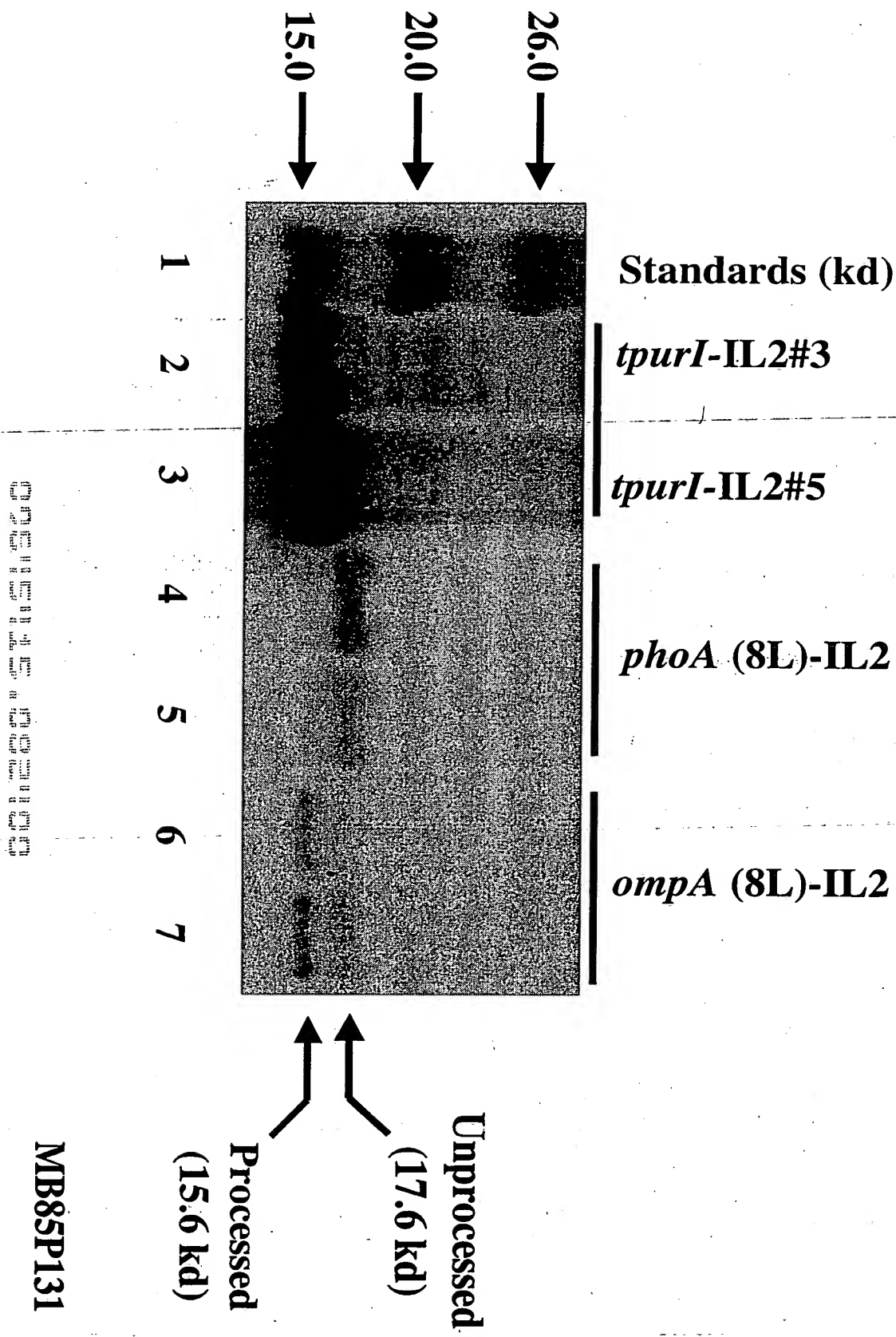


FIG. 9

ATG AAA CAG TCG ACT CTG GCG CTT CTG CTC TTG CTG TTA GCG CTG  
 M K Q S T L A L L L L L L A L  
 ACT AGT GTG GCC AAA GCG GCT CCT ACT AGC TCG AGC ACT AAG AAA  
 T S V A K A A P T S S S T K K  
 ACT CAA CTG CAA TTG GAG CAT CTG CTG CTG GAT CTG CAG ATG ATT  
 T Q L Q L E H L L L D L Q M I  
 CTG AAT GGC ATC AAT AAC TAC AAG AAC CCT AAG CTG ACT CGC ATG  
 L N G I N N Y K N P K L T R M  
 CTG ACT TTC AAA TTC TAC ATG CCG AAA AAG GCT ACC GAG CTC AAA  
 L T F K F Y M P K K A T E L K  
 CAT CTC CAG TGC CTG GAA GAG GAA CTG AAG CCG CTG GAG GAA GTA  
 H L Q C L E E E L K P L E E V  
 CTT AAC CTG GCA CAG TCT AAG AAC TTC CAC CTG CGT CCG CGT GAC  
 L N L A Q S K N F H L R P R D  
 CTG ATC TCC AAC ATC AAT GTA ATC GTT CTT GAG CTG AAG GGA TCC  
 L I S N I N V I V L E L K G S  
 GAA ACC ACC TTC ATG TGC GAA TAC GCT GAC GAA ACC GCC ACC ATT  
 E T T F M C E Y A D E T A T I  
 GTG GAG TTC CTG AAC CGT TGG ATC ACC TTT GCC CAA TCG ATC ATT  
 V E F L N R W I T F A Q S I I  
 AGC ACG TTA ACT TAA  
 S T L T \*

FIG. 10

# Antitumor efficacy of pTS-BrpTNF $\alpha$ Clone 2 in a staged Colon 38 tumor model.

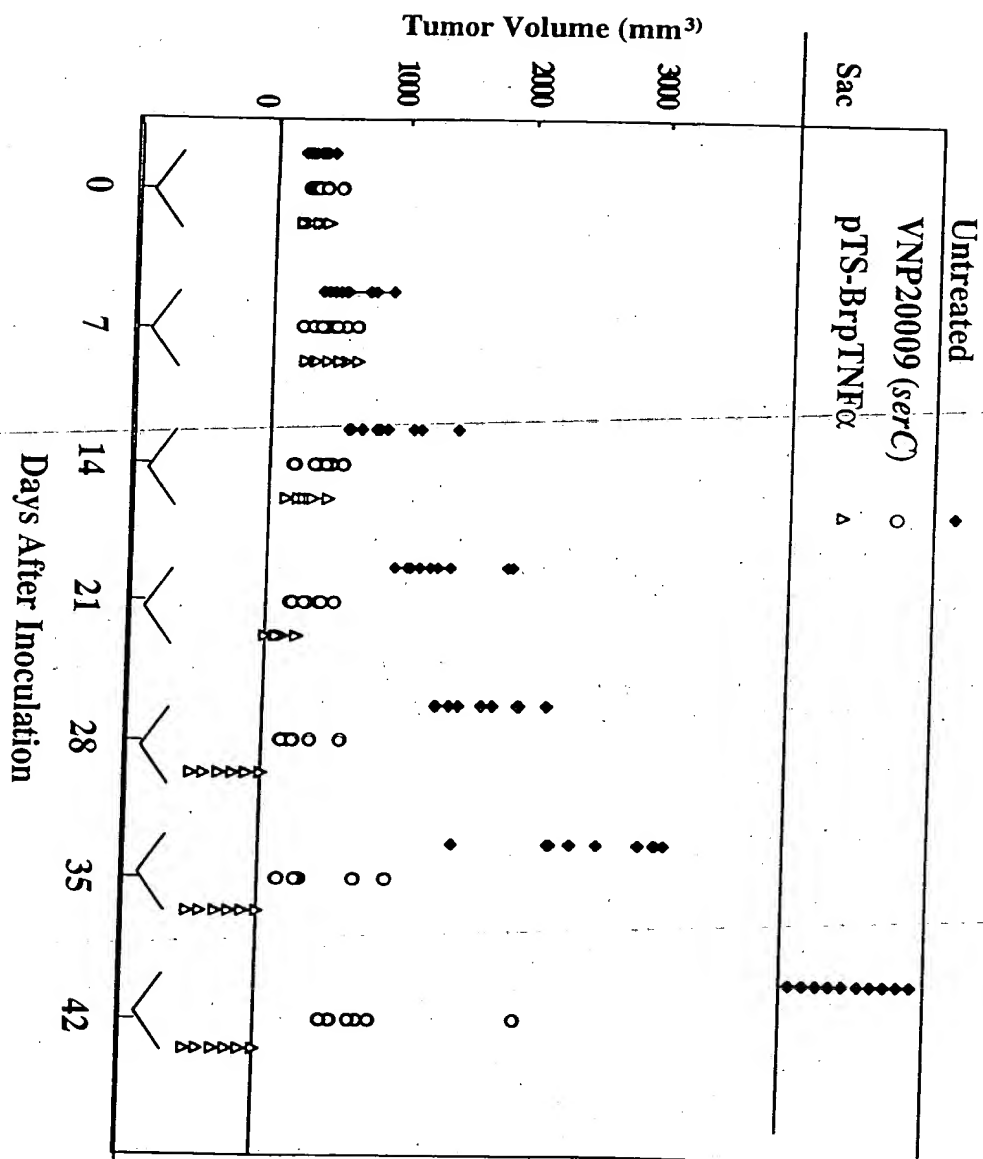


FIG. 11

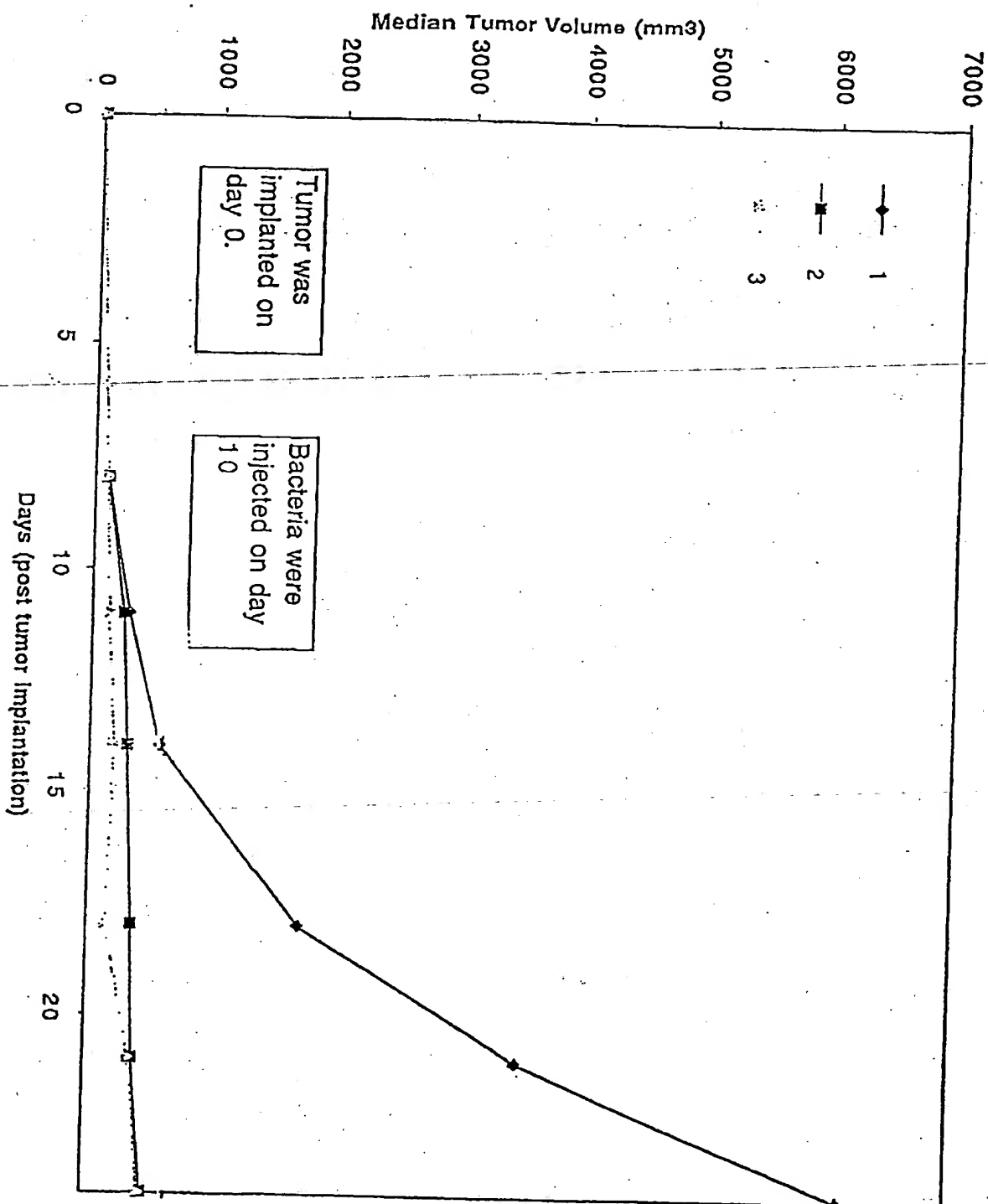


FIG. 12

$\beta$ -gal activity in strains carrying *pepT/bgal*

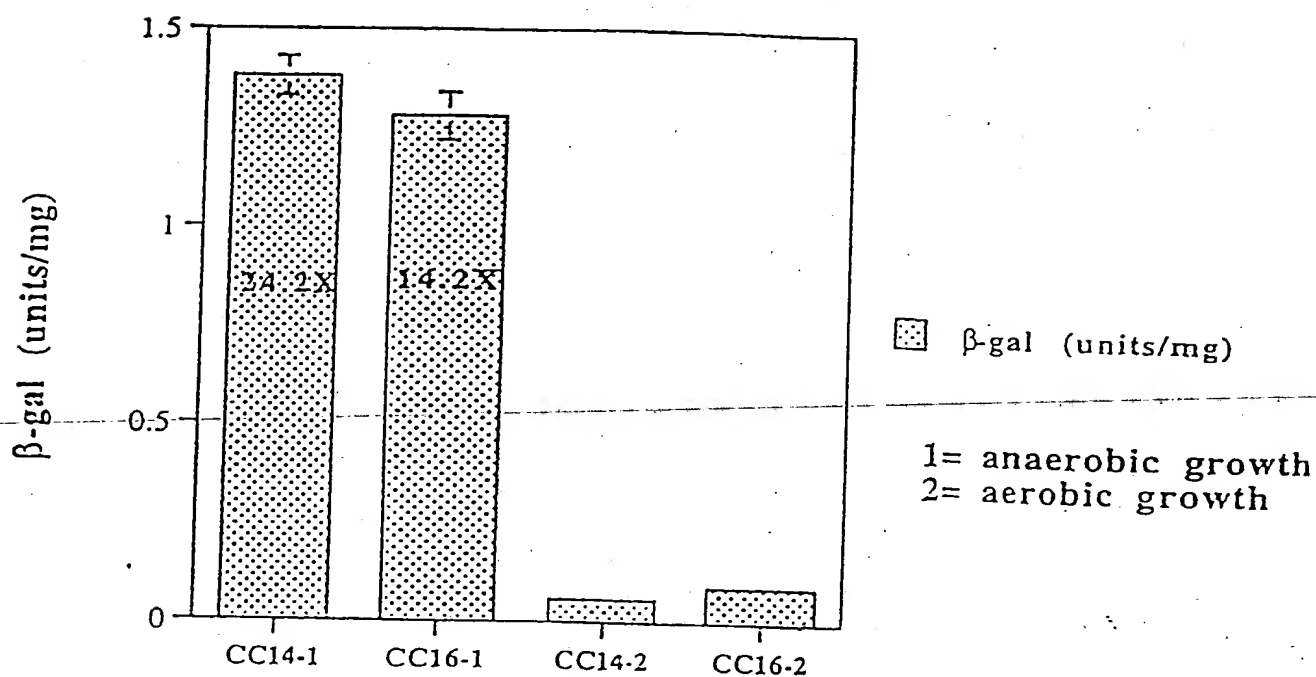


FIG. 13A

$\beta$ -gal activity *in vivo*, *pepT* $\beta$ -gal  $\pm$  BRP

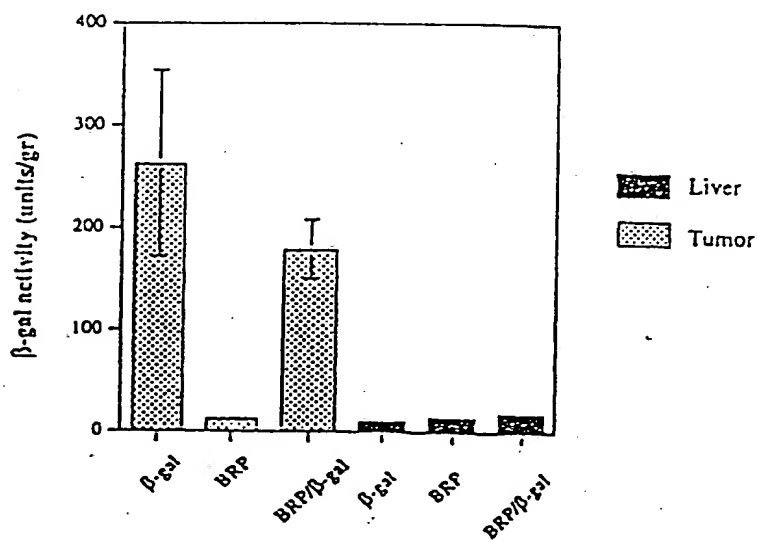


FIG. 13B

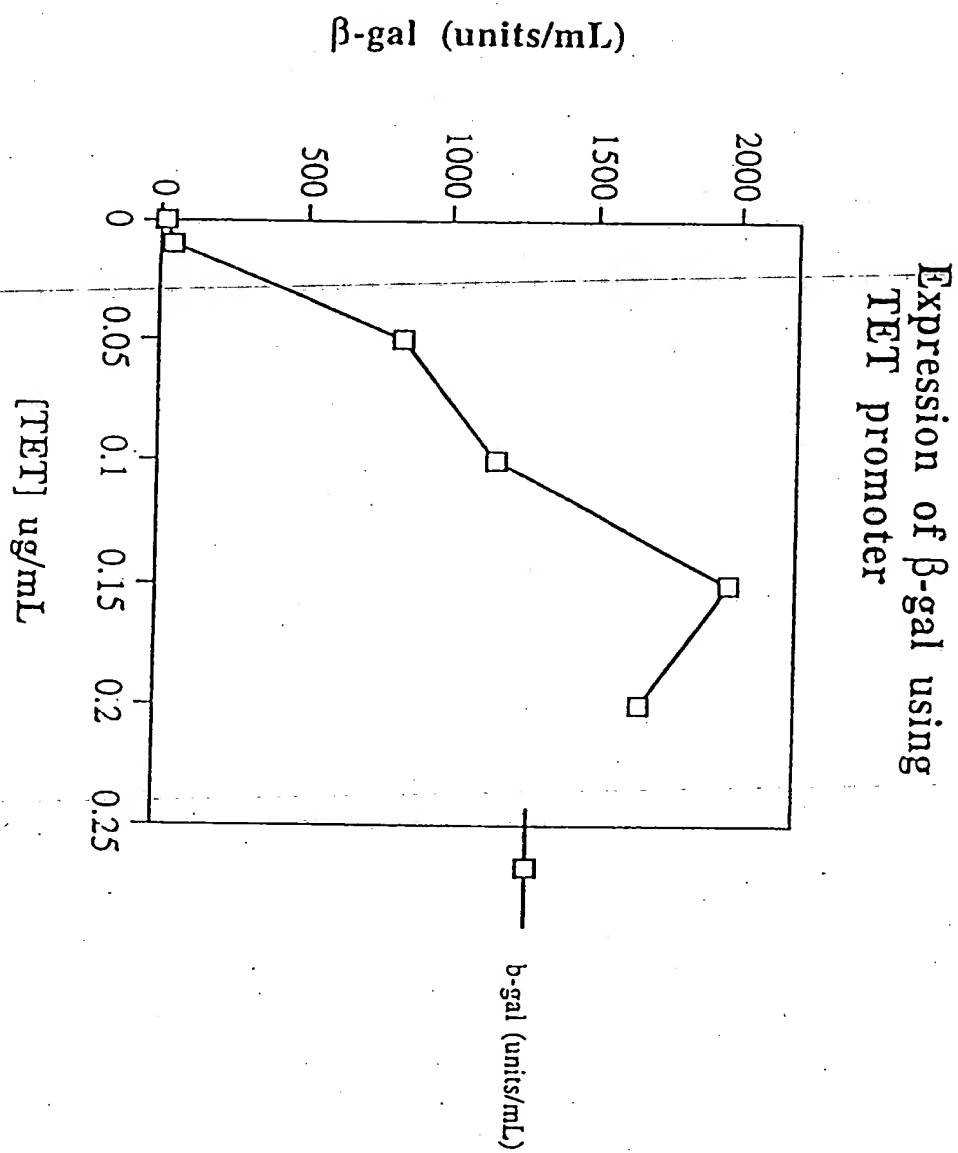
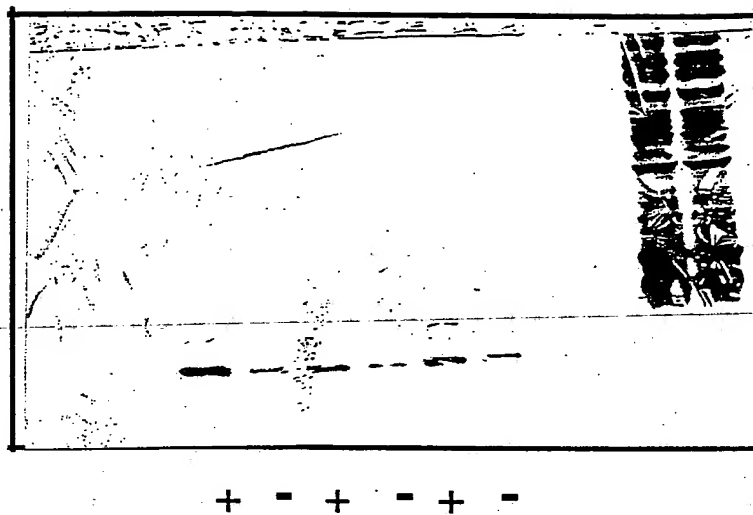


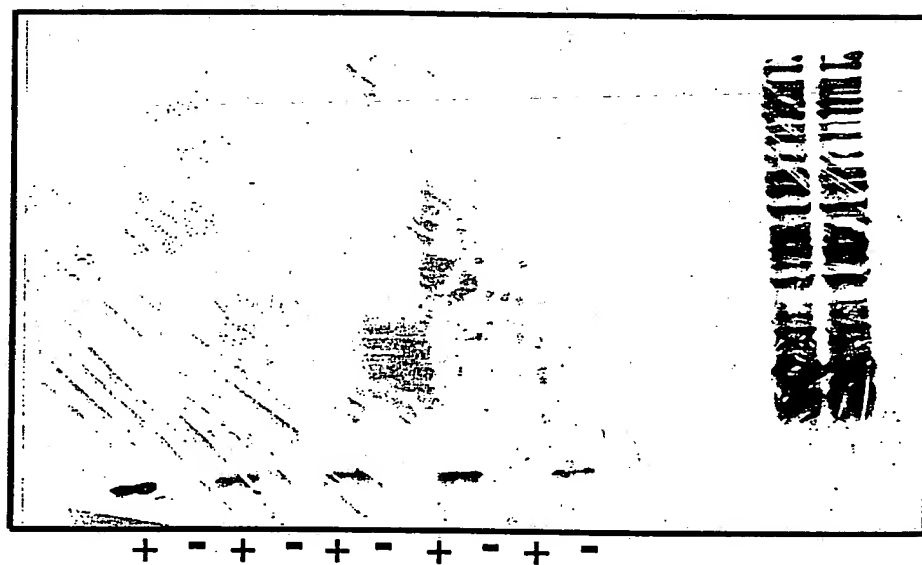
FIG. 14

**A.**

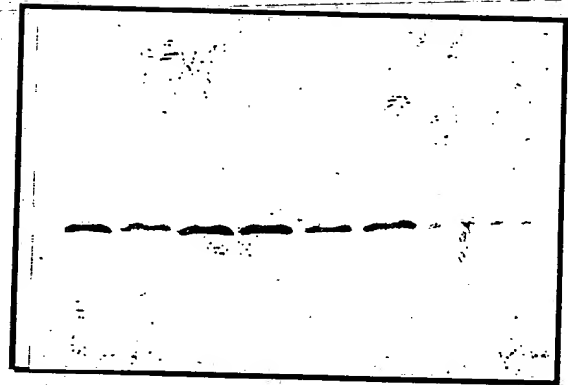
~25 kD →  
HexaHIS-endostatin

**B.**

~25 kD →  
HexaHIS-endostatin

**FIG. 15**

~25kD  
HexaHIS-endostatin



**FIG. 16**



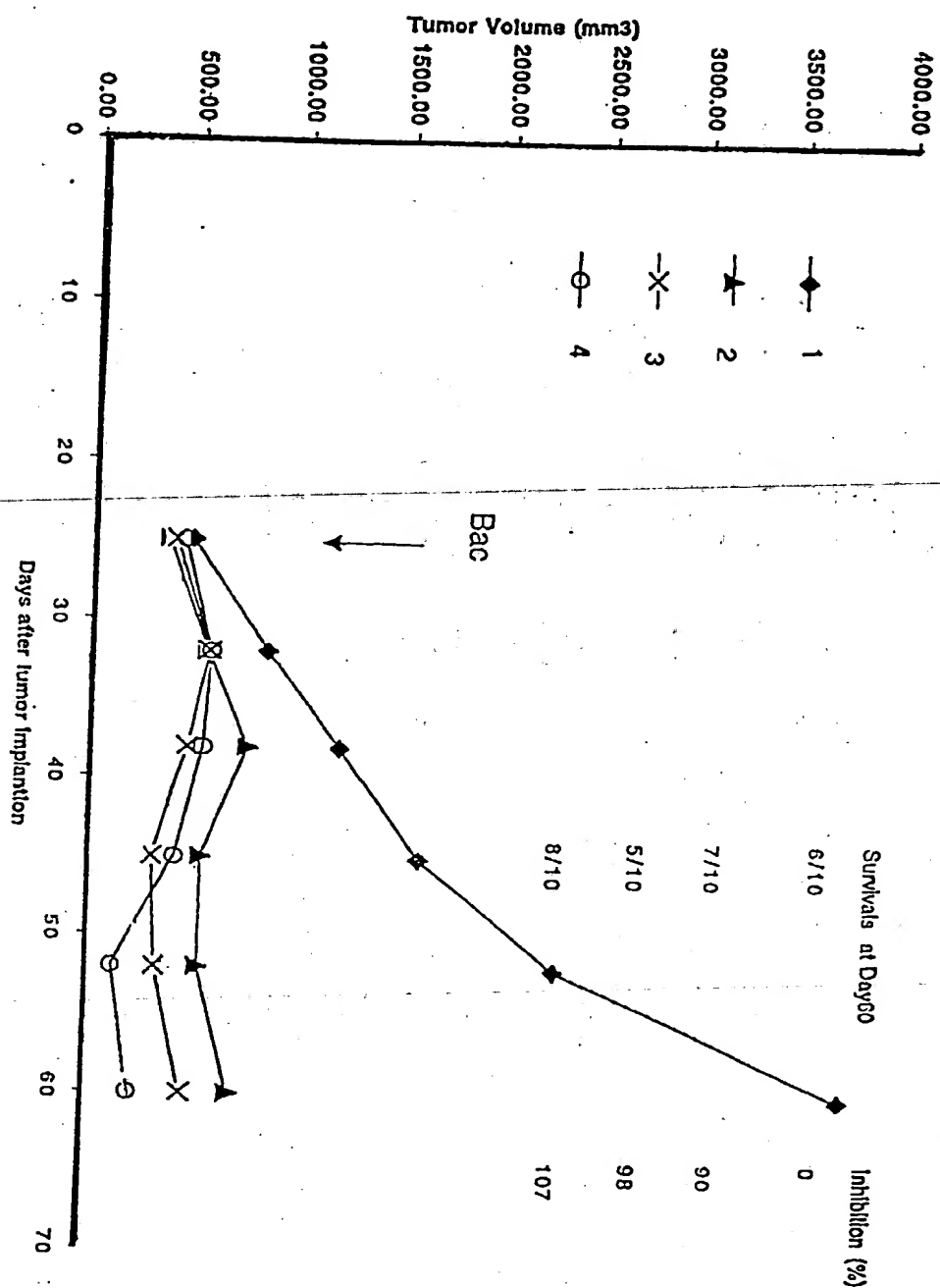


FIG. 17

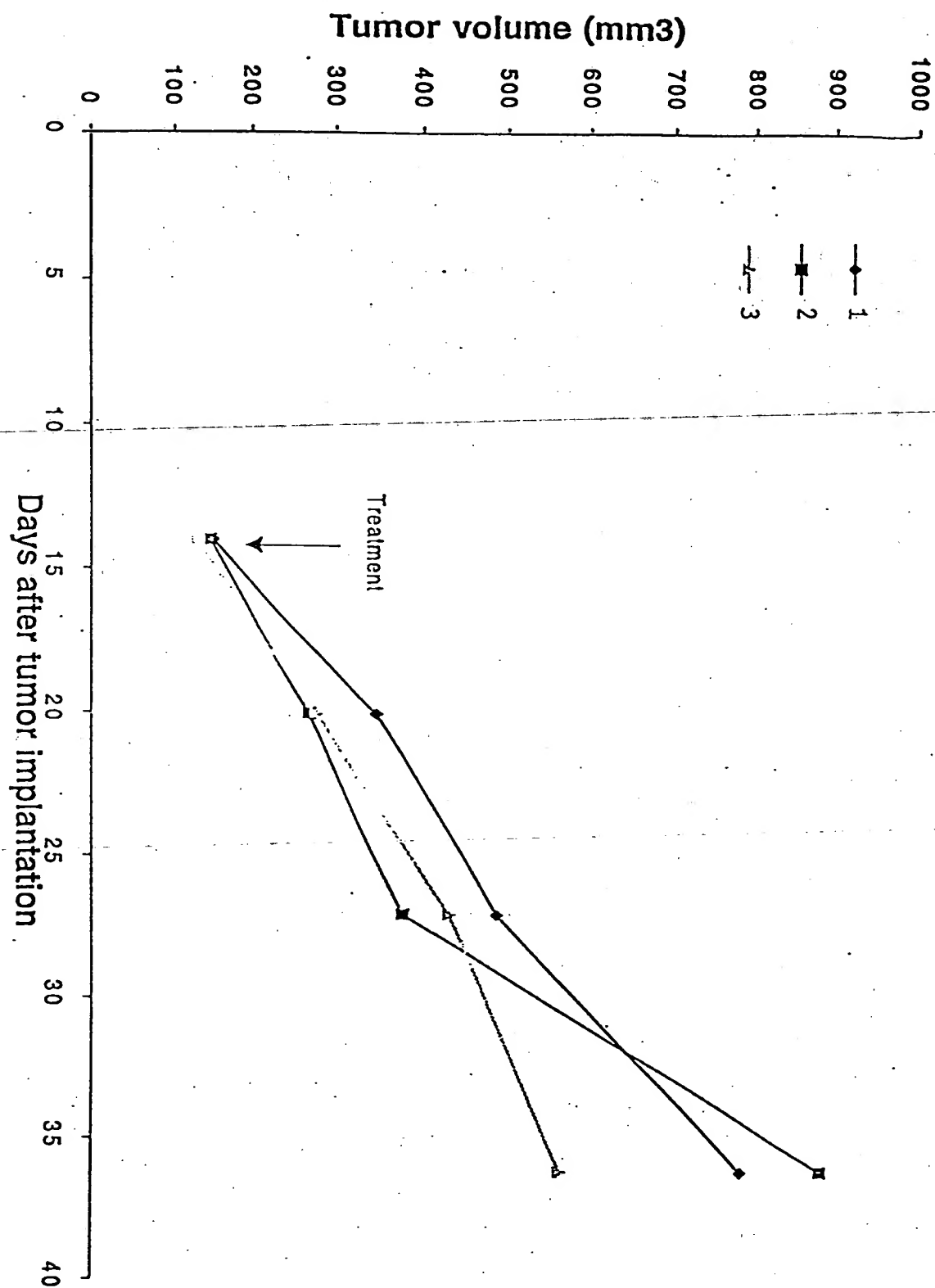
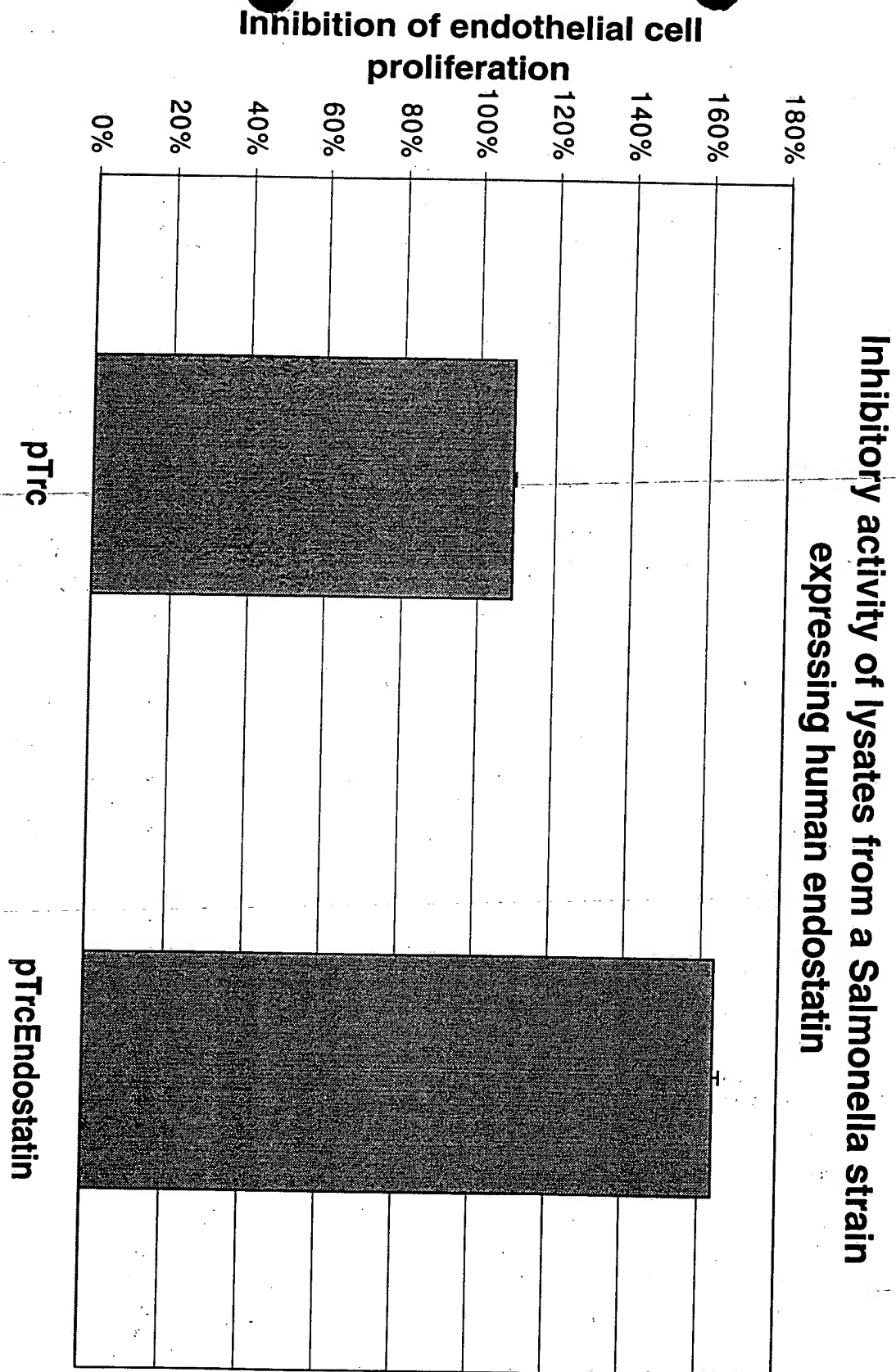
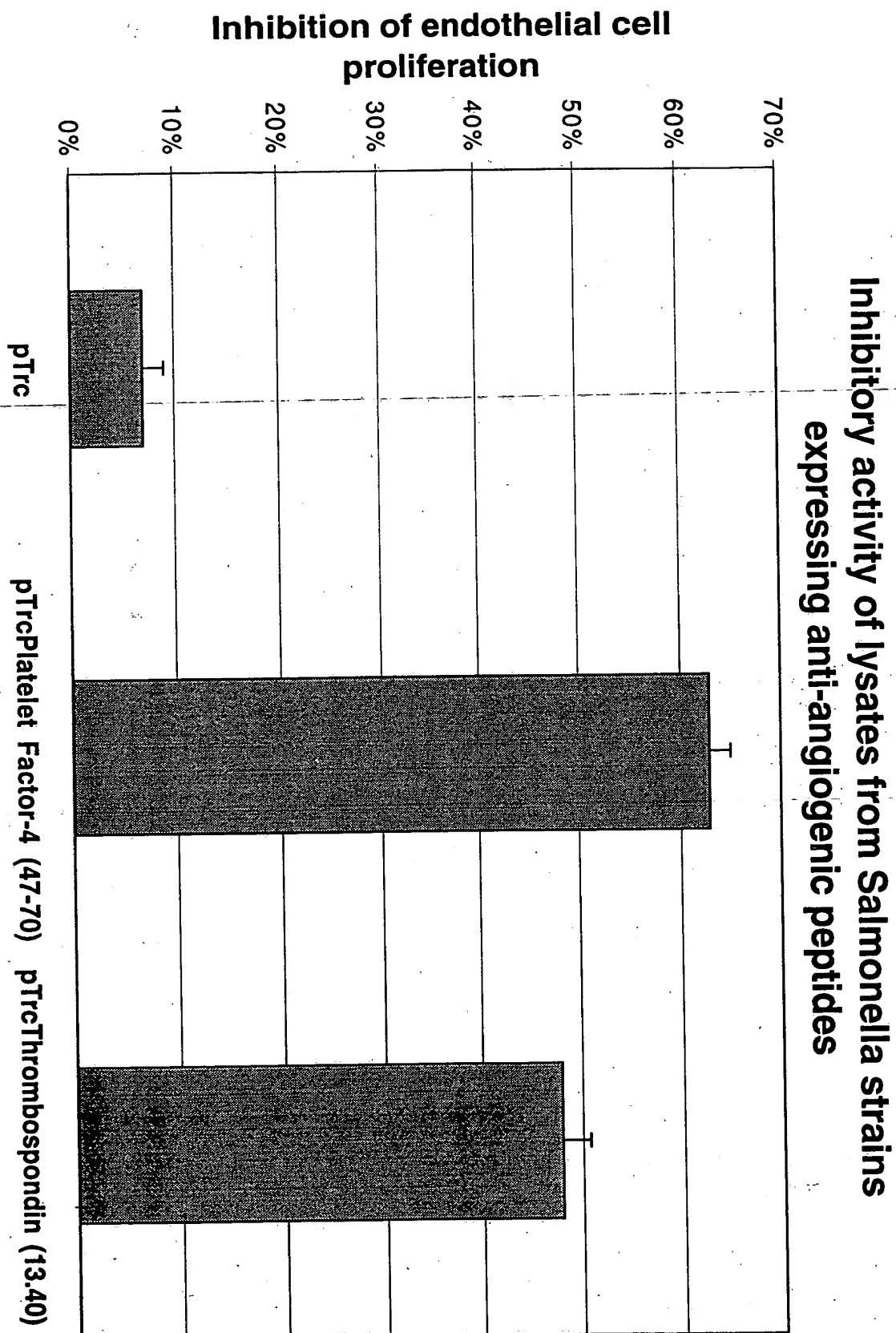


FIG. 18

**FIG. 19**

**FIG. 20**

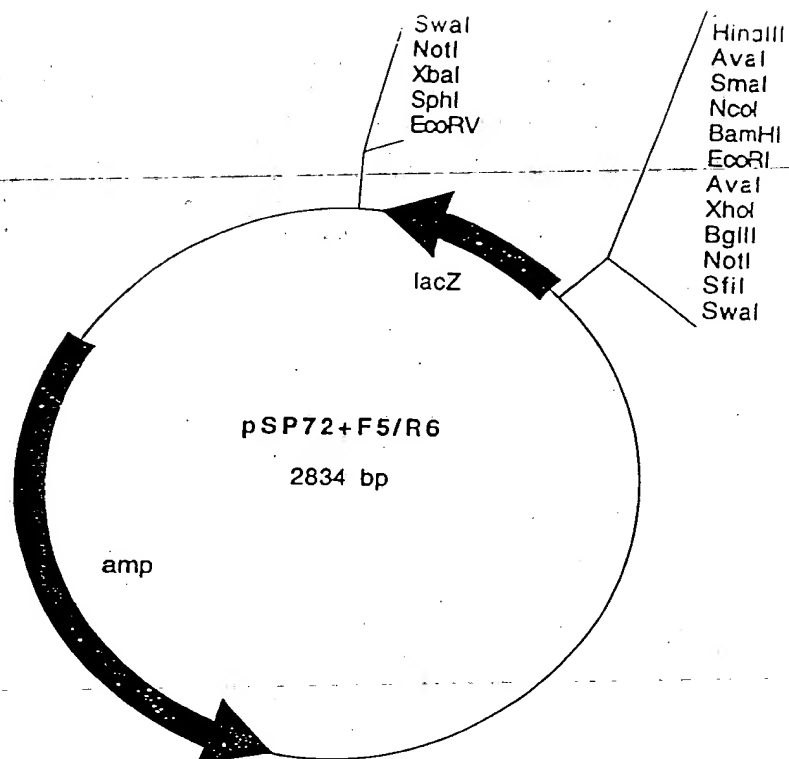


FIG. 21

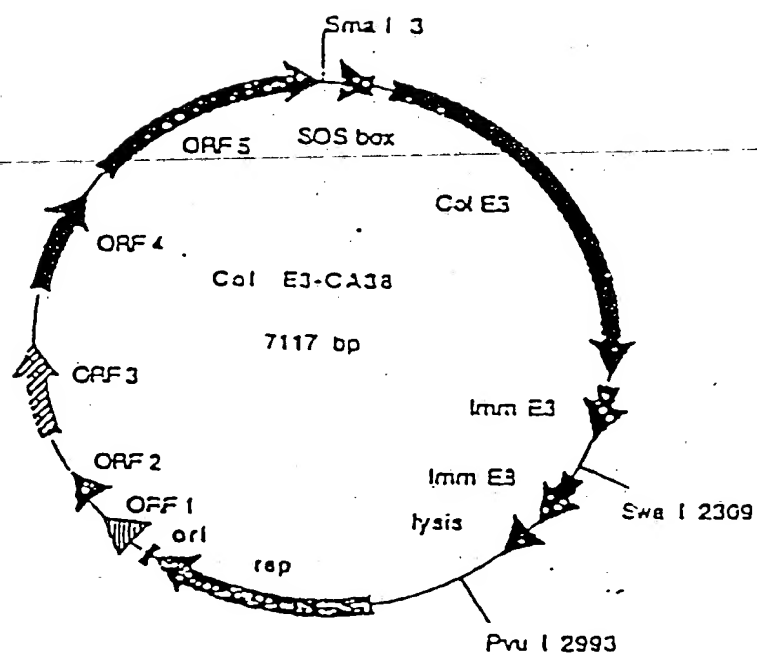
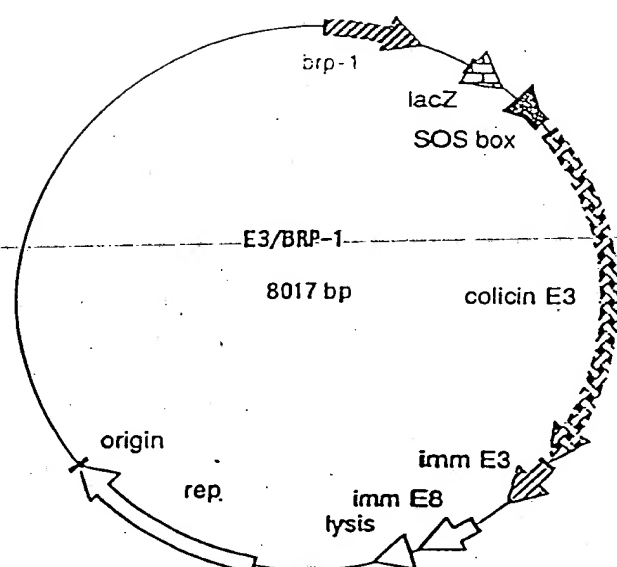







FIG. 22

**Key:**

-  BRP
-  LacZ alpha
-  colicin E3
-  immunity protein for E3
-  SOS box, SOS inducible promoter for E3

**FIG. 23**

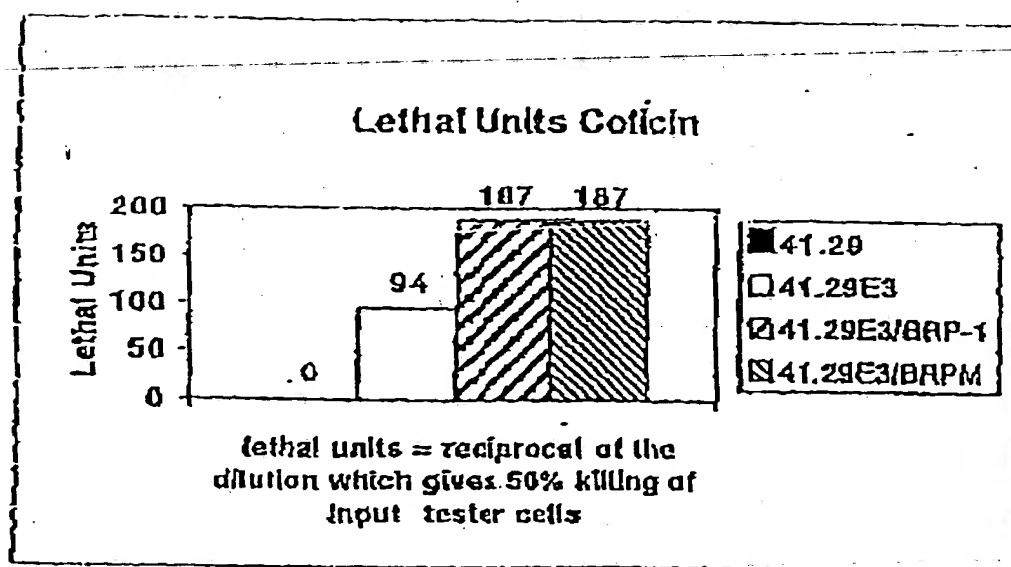


FIG. 24



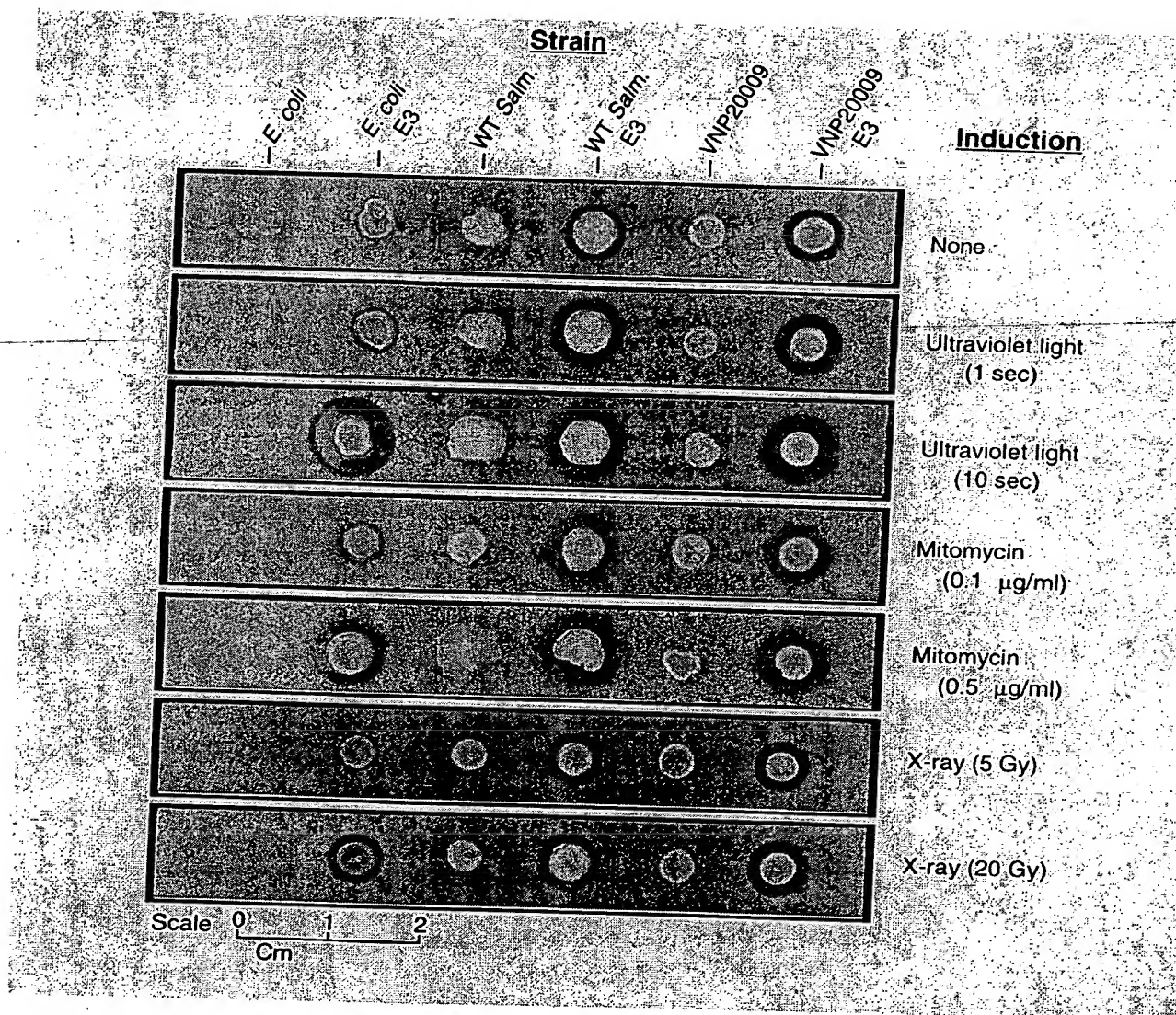


FIG. 25

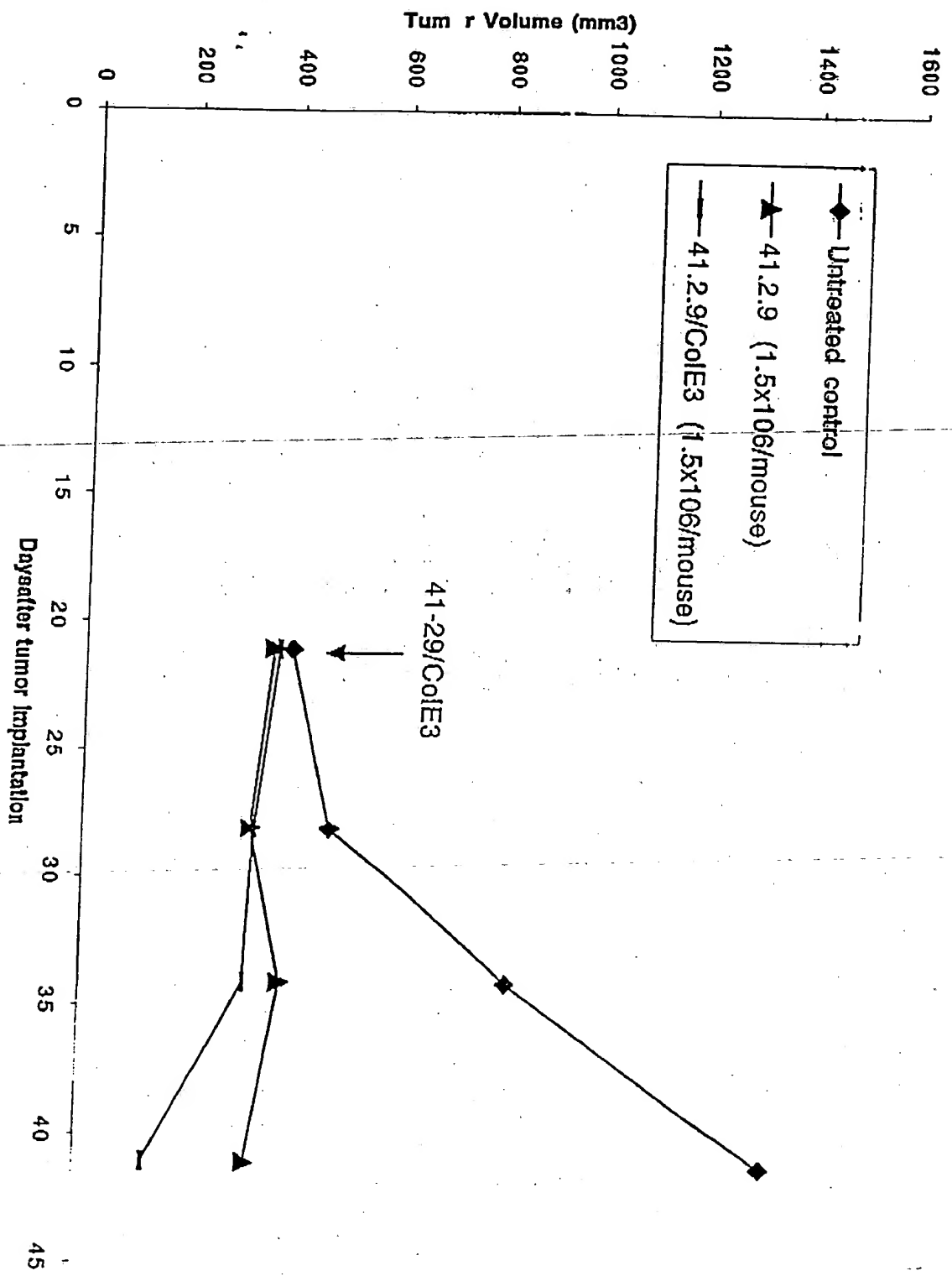


FIG. 26

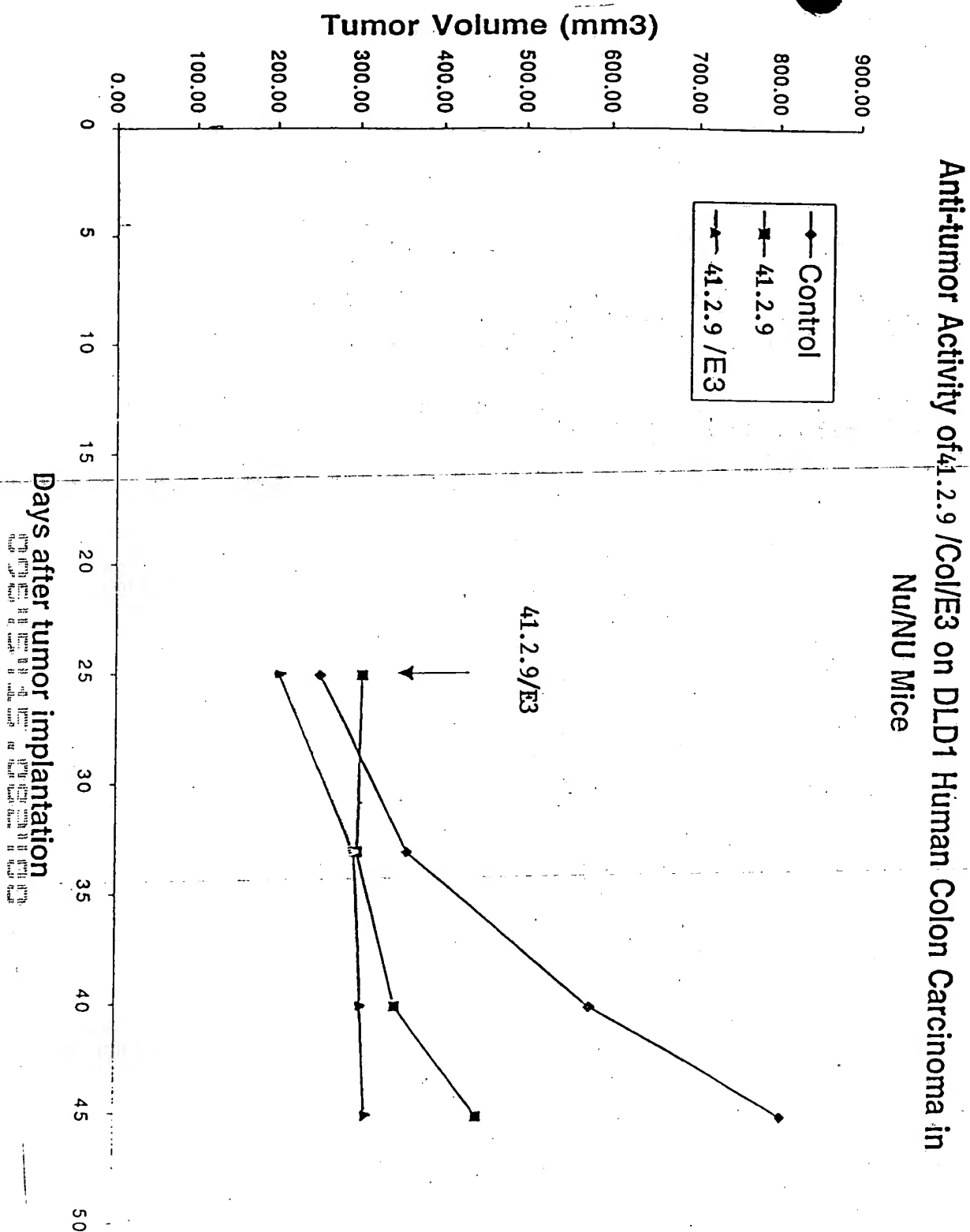


FIG. 27

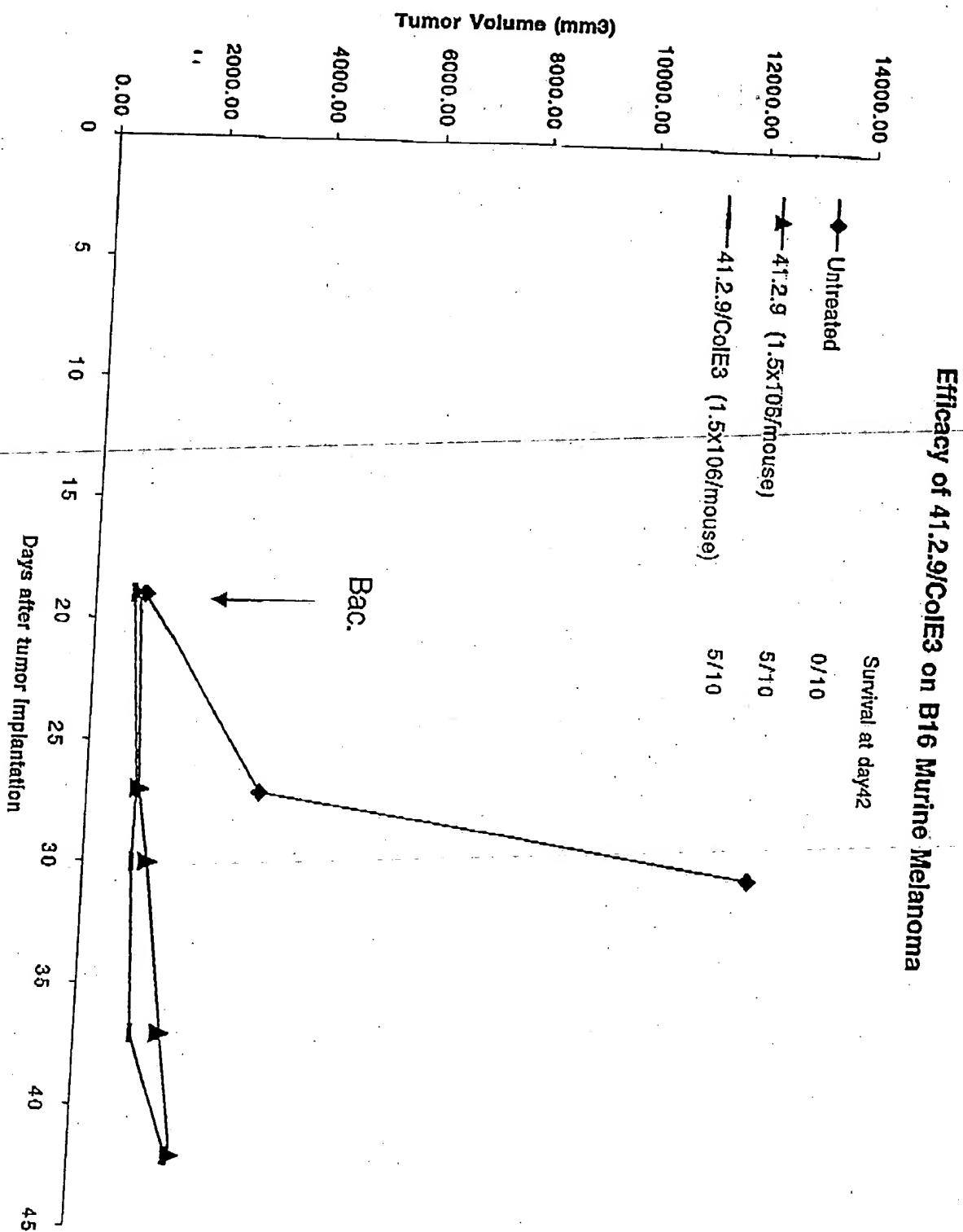
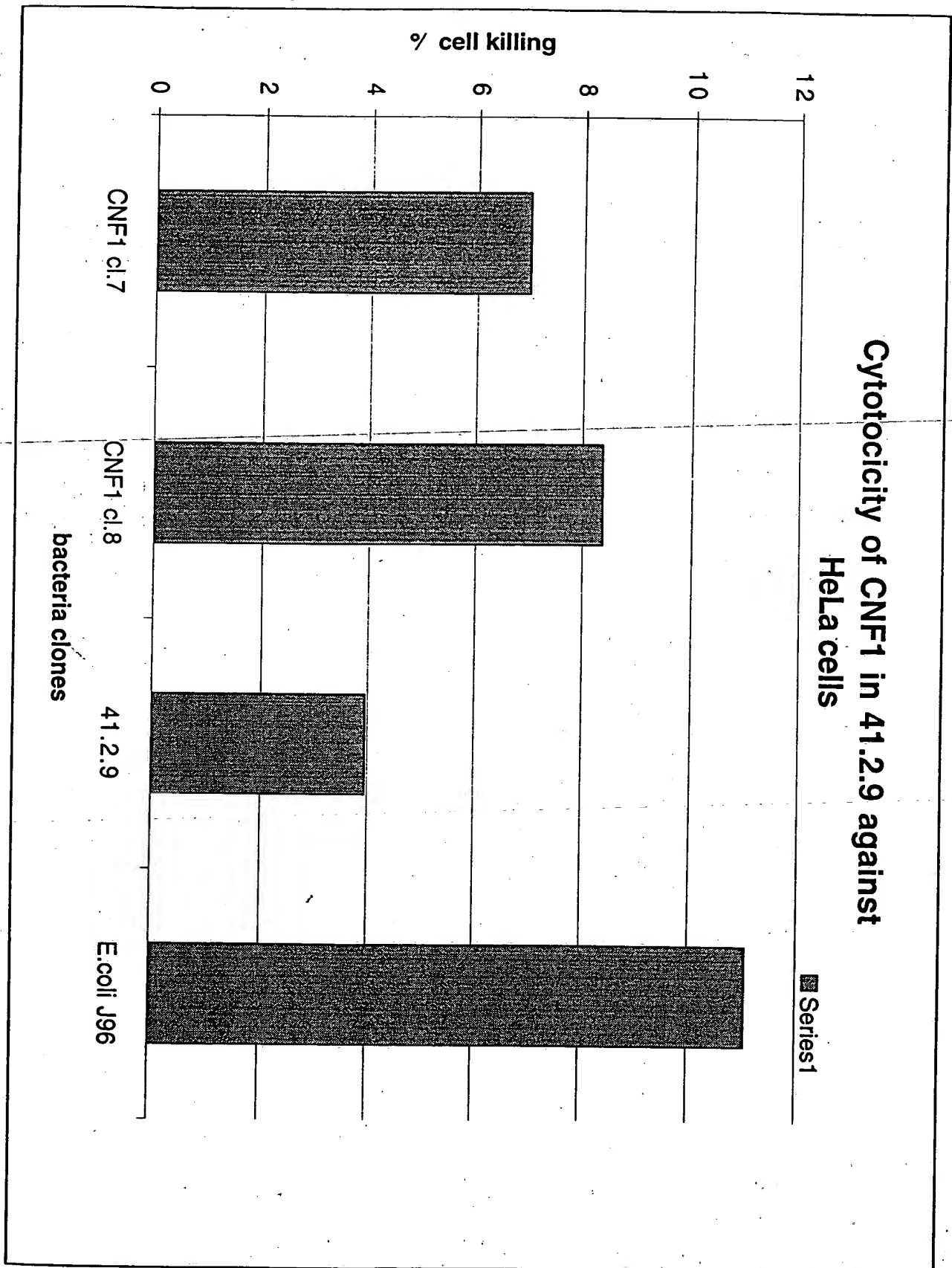
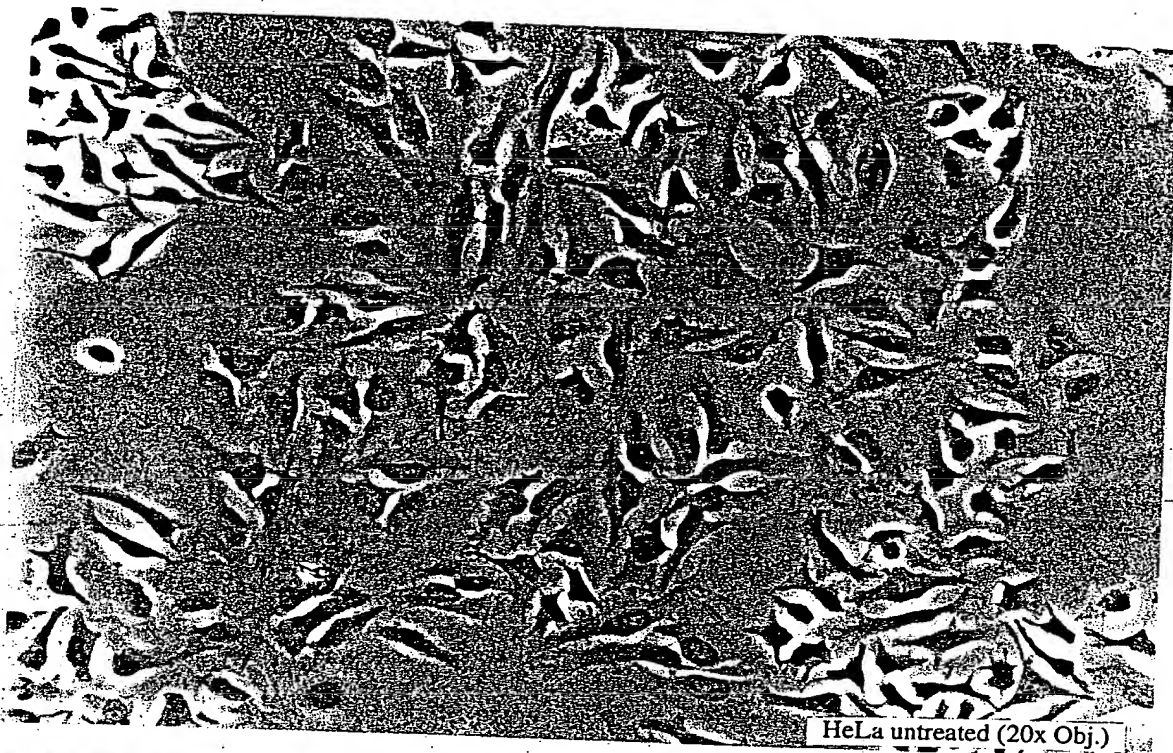


FIG. 28

**FIG. 29**

**A****B****FIG. 30**

GATATCATTC	TGGCCTCTGA	CGTTGTGATG	GTCGCACGTG	GCGATCTGGG	CGTTGAAATC	GGCGATCCGG	70
AGCTGGTTGG	TATCCAGAAA	GCGCTGATTC	GCCGTGCGCG	TCAGCTAAAC	CGCGCAGTCA	TCACCGCAAC	140
GCAAATGATG	GAGTCGATGA	TCACCAACCC	GATGCCGACC	CGTGCGGAAG	TGATGGACGT	GGCGAACGCC	210
GTCTTGGATG	GCACGGATGC	GGTTATGCTG	TCTGCCGAAA	CCGCAGCCGG	TCAGTATCCT	TCTGAAACCG	280
TTGCCGCAAT	GGCGCGCGTC	TGCCTGGGCG	CAGAAAAAAT	CCCCAGCATC	AATGTGTCTA	AACACCGTCT	350
CGACGTGCAG	TTGACAACG	TTGAAGAAGC	CATTGCCATG	TCTGCGATGT	ATGCGGCAAA	CCATCTGAAA	420
GGCGTTACCG	CGATCATCAC	CATGACGGAA	TCCGGTCGTA	CCGCGCTAAT	GACTTCCCGT	ATCAGCTCCG	490
GCCTGCCGAT	TTTCGCCATG	TCGCGCCATG	AACGCACGCT	GAACCTGACC	GCGCTCTATC	GCGGAGTAAC	560
GCCGGTGCA	TTTGATAGCG	CGGCTGATGG	CGTTGTGCGG	GCACATGAAG	CTGTTAATCT	GCTGCGCGAT	630
AAAGGGTATC	TGGTTTCCGG	CGACCTGGTT	ATCGTGACCC	AGGGCGATGT	CATGAGCACC	GTCGGTTCAA	700
CCAATACCAC	GCGGCGCGCC	CCTTAATTAA	CCCCGCATGC	GGGGGGCCAT	ATAGGCCGGG	GATTTAAATG	770
CAAACGTCCG	CCGAAACGCC	GACGCACTGT	GTTCCAGATA	TAGTCAAAAA	CCGGATTACC	CTGATTATGA	840
AACATCGCCG	CCATTTTTTG	CCCCTGAGAG	GCCATCAGCA	TGGCTGGAAT	GTCGACGCCC	CAGCCATGCG	910
GTACGAGAAA	AATGACTTTT	TCGTGTTTAC	GACGCATCTC	CTCGATAATC	TCCAGACCTT	CCCAGTCAAC	980
ACGCTGTTGA	ATTTTTTTTCG	GACCGCGCAT	CGCCAACCTA	GCCATCATCG	CCATTGCCTG	TGGCGCGGTG	1050
GCGAACATCT	CATCGACAAT	CGCTTCGCGC	TCAGCTTCGC	TACGCTGCGG	AAAGCACAAC	GACAGATTAA	1120
TTAGCGCCCG	GCGACGAGAA	CTCTTCCCCA	GCCGTCCGGC	AAAACGCCCC	AGCGTCGCCA	GCAAAGGGTC	1190
GCGGAATGAT	GCCGGTGTTA	ATGCGATCCC	CGCCATTGCC	GCCGCGCCCA	ACCAGGCGCC	CCAATACTGT	1260
GGATAGCGAA	AGGATTTTTC	GAATTCAGGG	ATATACTCAC	TATTATTTT	TTTGGTTTCC	ATGCTTTTCC	1330
AGGGTCTGCT	GACGCGAAAA	GGAATGTGA	ATAGTGTAGC	GACGTCTGCG	TCTCACACAA	AACAAAAAAG	1400
CGGCGACACA	TCGCGTACCG	GCTCTGTCAG	CGCATTTGTT	AATCGAAGCG	CAGTTGCGGC	AGAACCTCTT	1470
TCACCTGTGC	CAGGTATTCA	CGACGATCTG	ACCCCGTCAG	ACCTTCGCTG	CGCGGCAATT	TTGCTGTCAG	1530
AGGGTTAACG	GCTTGCTGGT	TGATC					1555

FIG. 31

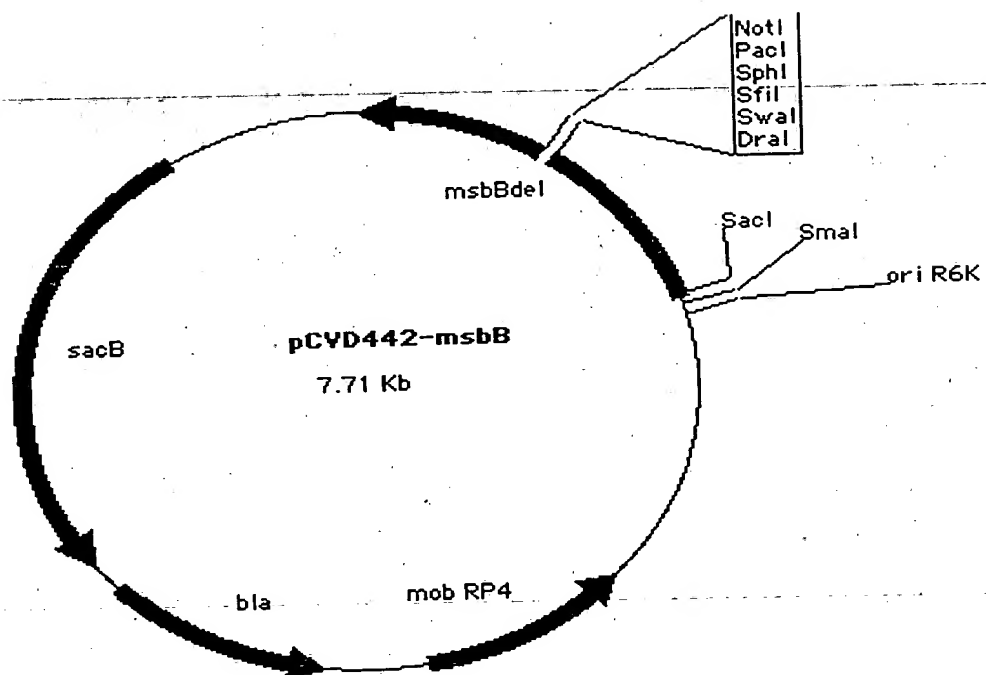


FIG. 32



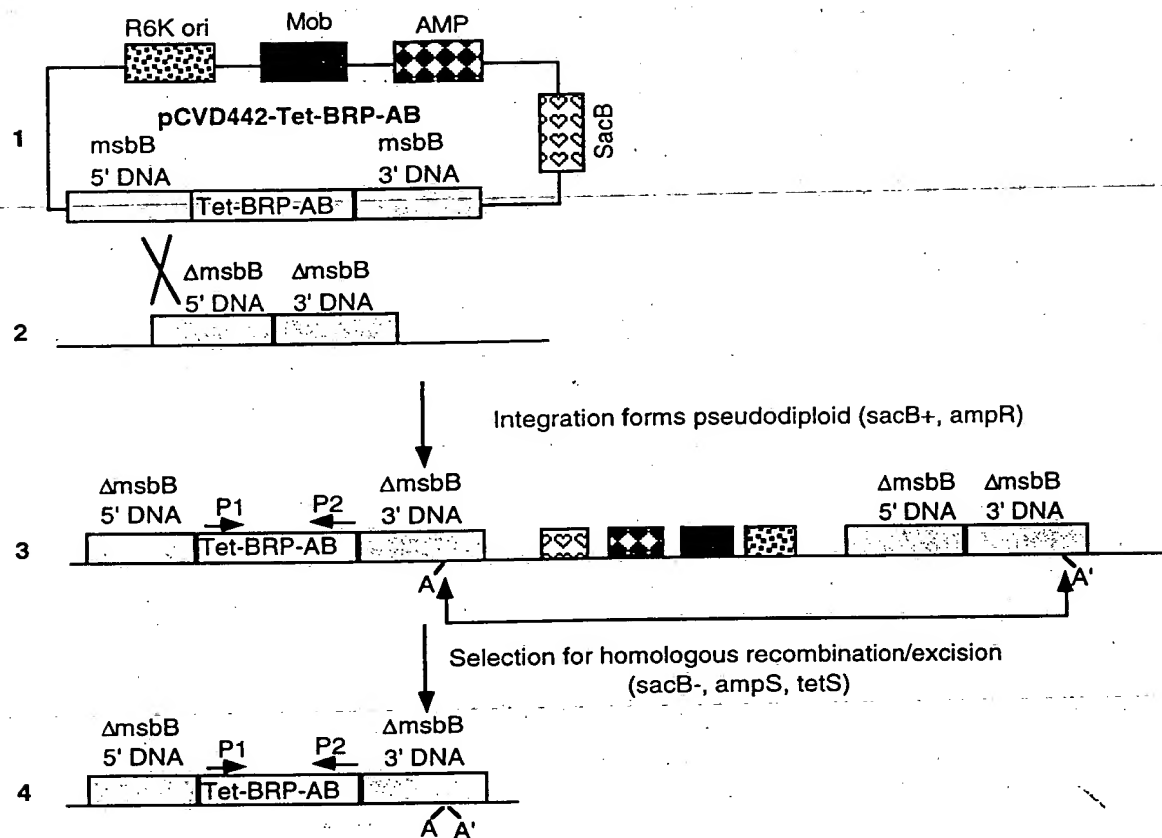


FIG.33

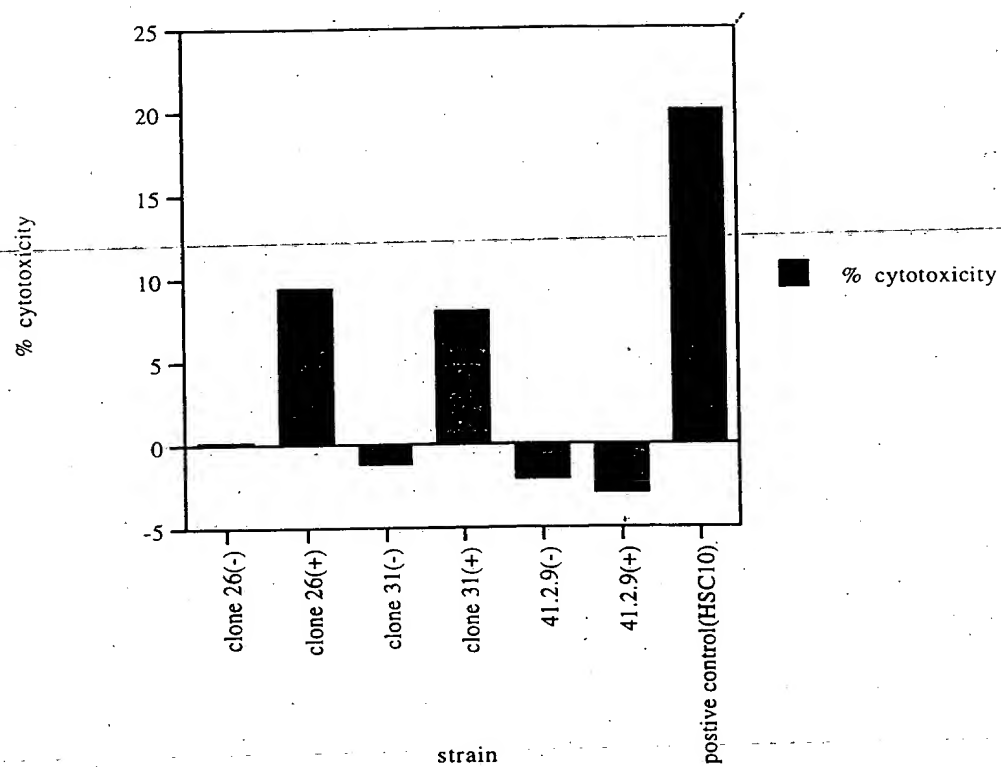


FIG. 34

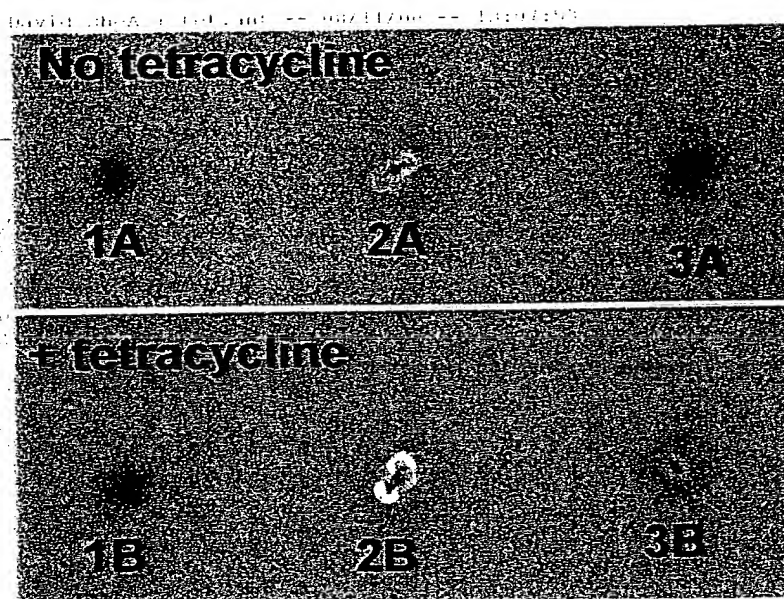


FIG. 35

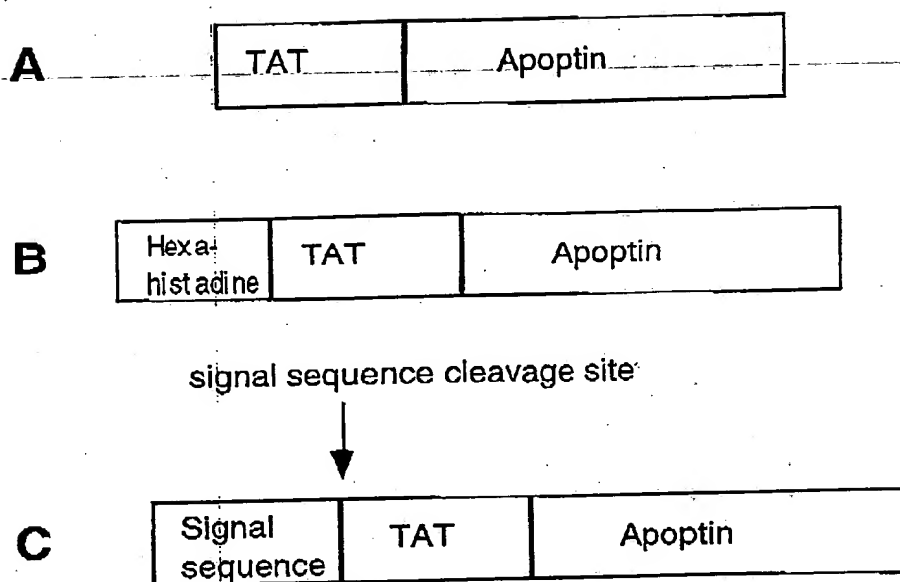


FIG. 36

## Protein Sequence of 616-4 F

page 1

Length of 616-4 F: 551 bp; Listed from: 1 to: 551;  
 Translated from: 7 to: 409 (Entire region);  
 Genetic Code used: Universal; Wed, Aug 16, 2000 1:40 PM

Frame 1

M	A	Y	G	R	K	K	R	R	Q	R	R	R	M	N				
NAG	ACC	ATG	GCT	TAT	GGC	AGA	AAA	AAA	AGA	AGA	CAG	AGA	AGA	ATG	AAC			
	9			18			27		36			45						
A	L	Q	E	D	T	P	P	G	P	S	T	V	F	R	P	P	T	S
GCG	CTG	CAG	GAA	GAT	ACC	CCG	CCG	GGC	CCG	TCC	ACC	GTG	TTT	CGC	CCG	CCG	ACC	TCC
	60			69			78			87			96				105	
S	R	P	L	E	T	P	H	C	R	E	I	R	I	G	I	A	G	I
TCC	CGC	CCG	CTG	GAA	ACC	CCG	CAT	TGC	CGC	GAA	ATC	CGC	ATC	GGC	ATC	CGC	GGC	ATC
	117			126			135			144			153				162	
T	I	T	L	S	L	C	G	C	A	N	A	R	A	P	T	L	R	S
ACC	ATC	ACC	CTG	TCC	CTG	TGC	GGC	TGC	GCG	AAC	GCG	CGC	GCG	CCG	ACC	CTG	CGC	TCC
	174			183			192			201			210				219	
A	T	A	D	N	S	E	N	T	G	F	K	N	V	P	D	L	R	T
GCG	ACC	GCG	GAT	AAC	TCC	GAA	AAC	ACC	GGC	TTT	AAA	AAC	GTC	CCG	GAT	CTG	CGC	ACC
	231			240			249			258			267				276	
D	Q	P	K	P	P	S	K	K	R	S	C	D	P	S	E	Y	R	V
GAT	CAG	CCG	AAA	CCG	CCG	TCC	AAA	AAA	CGC	TCC	TGC	GAT	CCG	TCC	GAA	TAT	CGC	GTC
	288			297			306			315			324				333	
S	E	L	K	E	S	L	I	T	T	T	P	S	R	P	R	T	A	R
TCC	GAA	CTG	AAA	GAA	TCC	CTG	ATC	ACC	ACC	ACC	CCG	TCC	CGC	CCG	CGC	ACC	GCC	CGC
	345			354			363			372			381				390	
R	C	I	R	L														
CGC	TGC	ATC	CGC	CTC	TGA	AAG	CTT	GGC	TGT	TTT	GGC	GGA	TGA	GAG	AAG	ATT	TTC	AGC
	402			411			420			429			438				447	
CTG	ATA	CAG	ATT	AAA	TCA	GAA	CGC	AGA	AGC	GGT	CTG	ATA	AAA	CAG	AAT	TTG	CCT	GGC
	459			468			477			486			495				504	
GGC	AGT	AGC	GCG	GTG	GTC	CCA	CCT	GAC	CCC	ATG	CCG	AAC	TCA	GA				
	516			525			534			543								

FIG. 37

## Protein Sequence of TAP6H8 trcF

page 1

Length of TAP6H8 trcF: 751 bp; Listed from: 1 to: 444;  
 Translated from: 7 to: 427 (Entire region);  
 Genetic Code used: Universal; Mon, Aug 14, 2000 3:19 PM

Frame 1

```

      M  A  H  H  H  H  H  H  Y  G  R  K  K  R  R
NAG ACC ATG GCT CAT CAC CAT CAC CAC CAT TAT GGC CGC AAA AAA CGC CGT
      9      18      27      36      45

      Q  R  R  R  M  N  A  L  Q  E  D  T  P  P  G  P  S  T  V
CAG CGC CGT CGC ATG AAG GCG CTG CAG GAA GAT ACC CCG CCG GGC CGC TCC ACC GTG
      60      69      78      87      96      105

      F  R  P  R  T  S  S  R  P  L  E  T  P  H  C  R  E  I  R
TTT CGC CCG CCG ACC TCC TCC CGC CCG CTG GAA ACC CCG CAT TGC CGC GAA ATC CGC
      117      126      135      144      153      162

      I  G  I  A  G  I  T  I  T  L  S  L  C  G  C  A  N  A  R
ATC GGC ATC GCG GGC ATC ACC ATC ACC CTG TCC CTG TGC GGC TGC GCG AAC GCG CGC
      174      183      192      201      210      219

      A  P  T  L  R  S  A  T  A  D  N  S  E  N  T  G  F  K  N
GCG CCG ACC CTG CGC TCC GCG ACC GCG GAT AAC TCC GAA AAC ACC GGC TTT AAA AAC
      231      240      249      258      267      276

      V  P  D  L  R  T  D  Q  P  K  P  P  S  K  K  R  S  C  D
GTC CCG GAT CTG CGC ACC GAT CAG CCG AAA CCG CCG TCC AAA AAA CGC TCC TGC GAT
      288      297      306      315      324      333

      F  S  E  Y  R  V  S  E  L  K  E  S  L  I  T  T  T  P  S
CCG TCC GAA TAT CGC GTC TCC GAA CTG AAA GAA TCC CTG ATC ACC ACC ACC CCG TCC
      345      354      363      372      381      390

      R  P  R  T  A  R  R  C  I  R  L
CGC CCG CGC ACC GCC CGC CGC TGC ATC CGC CTC TGA AAG CTT GGC TGT TTT
      402      411      420      429      438

```

FIG. 38

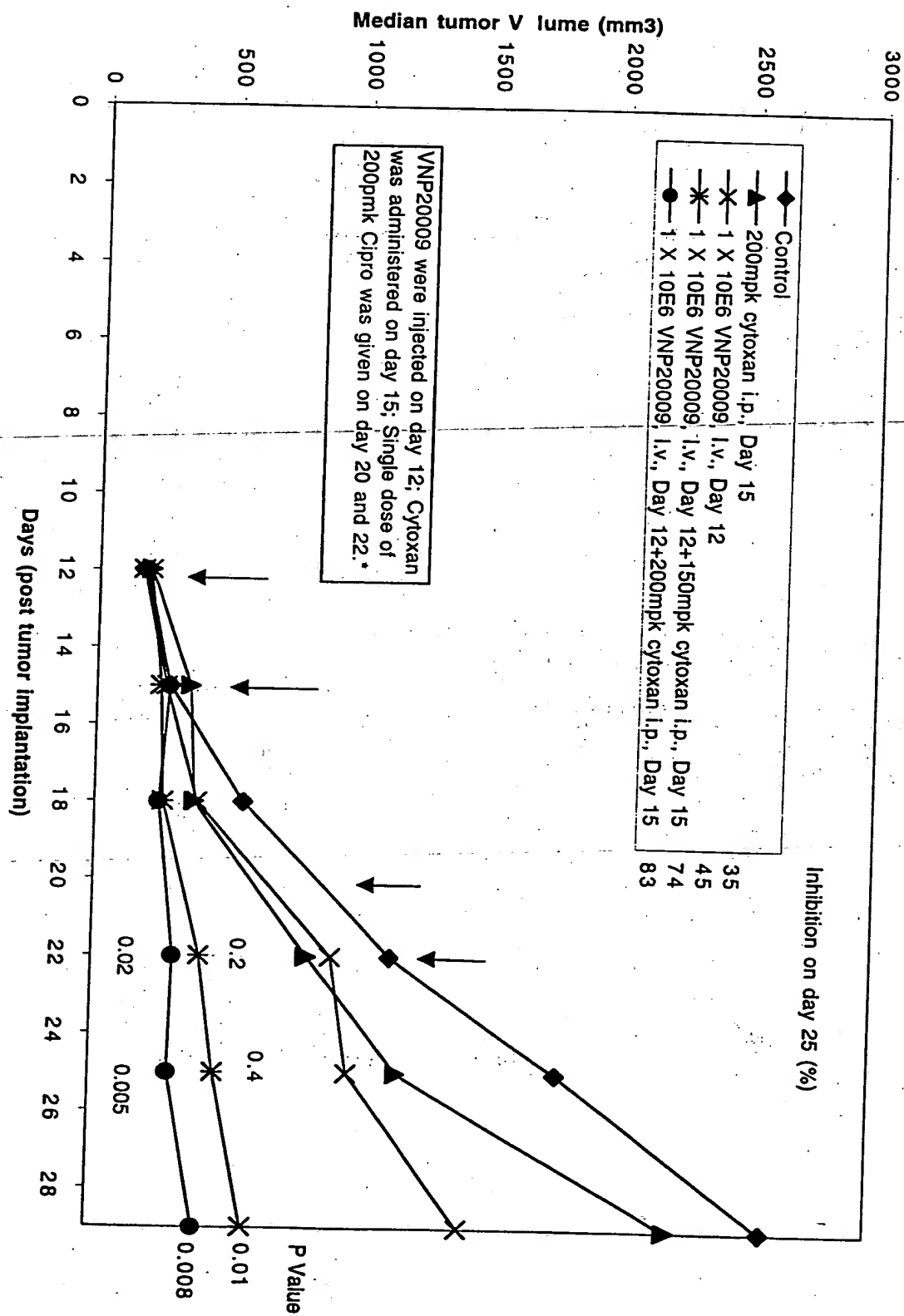


FIG. 39

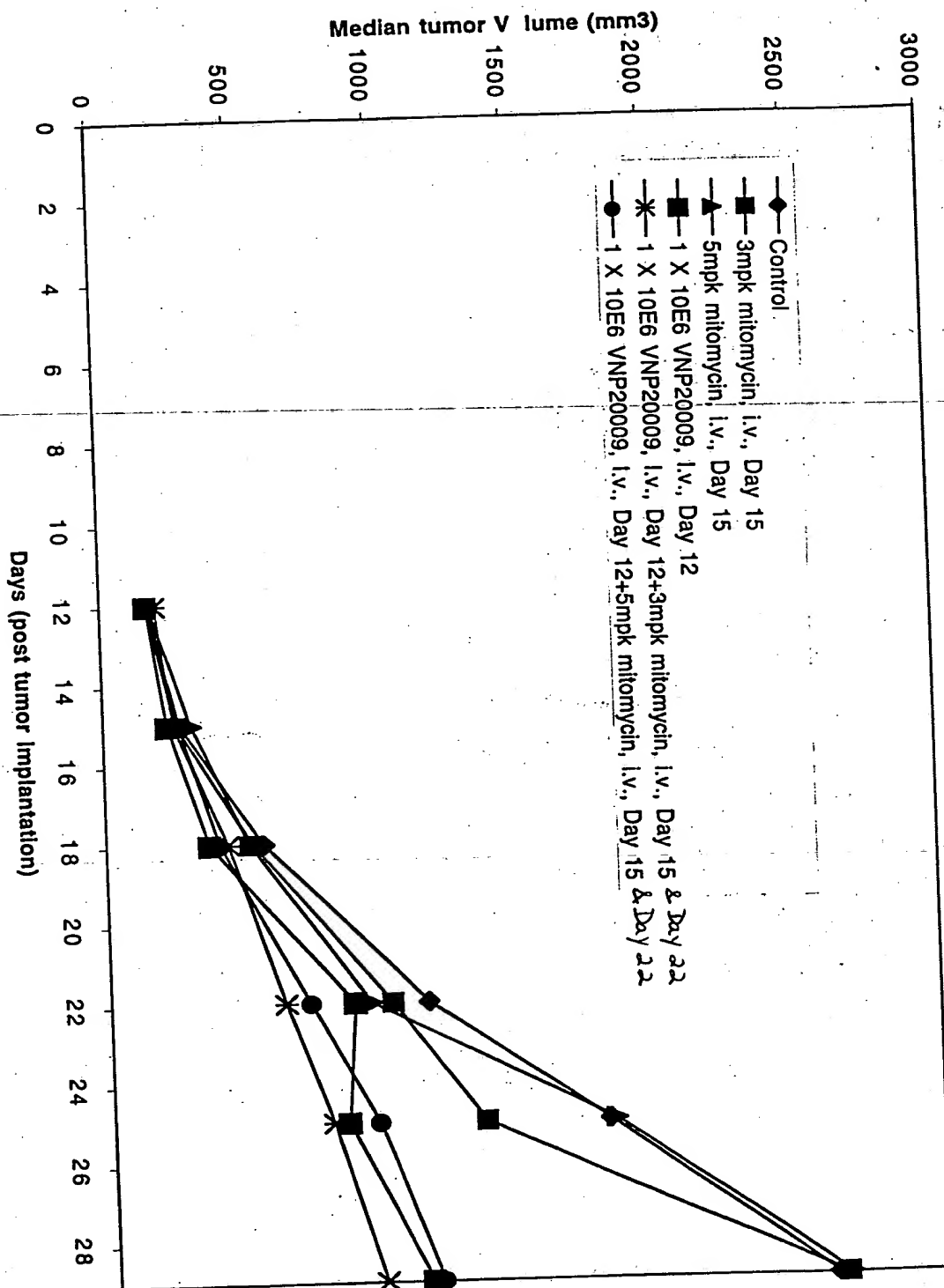


FIG. 40



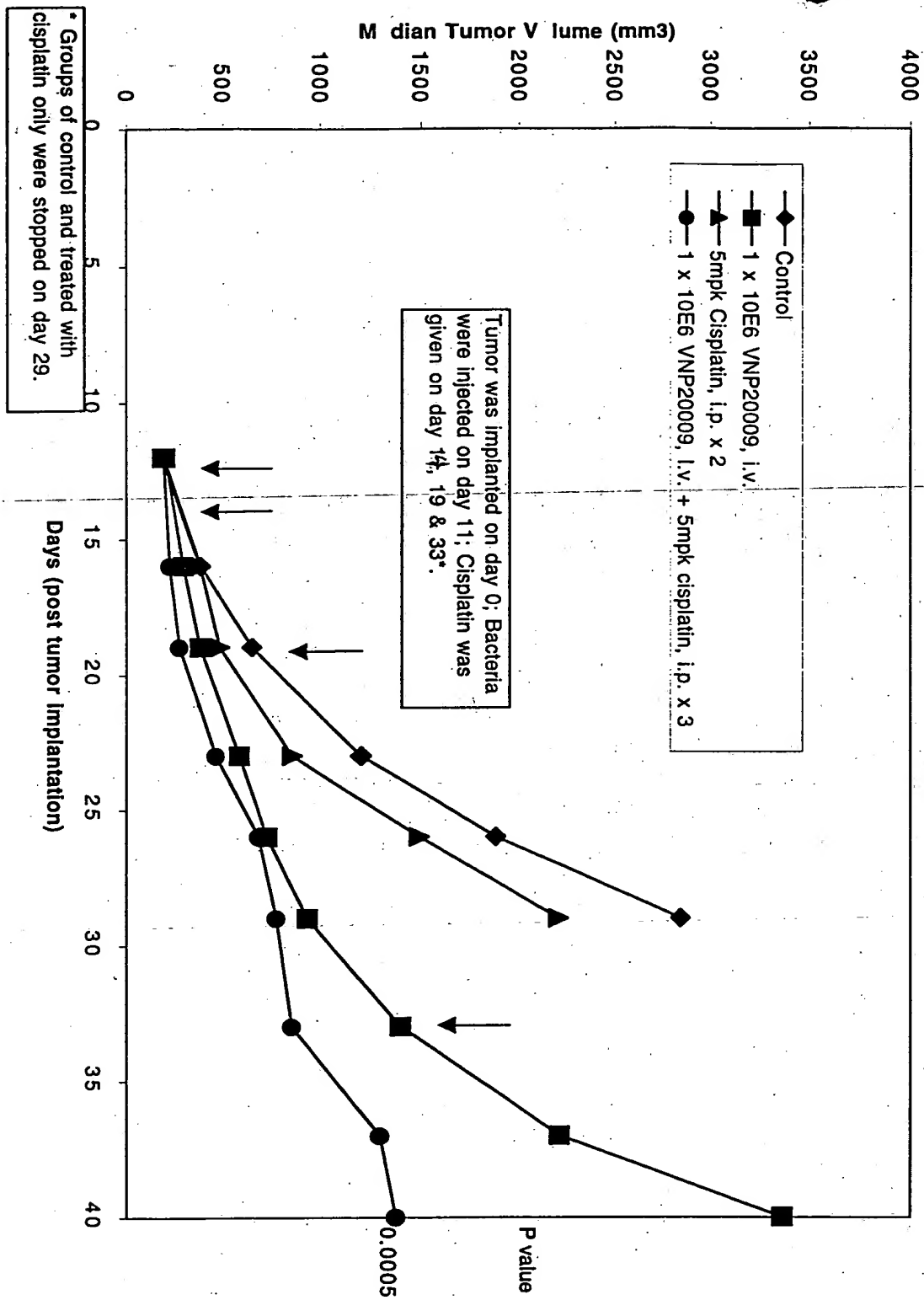


FIG. 41